JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVII WASHINGTON, D. C., APRIL 15, 1919 NO. 1

MEAT EXTRACTS, THEIR COMPOSITION AND IDENTIFICATION

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INTRODUCTION

The historical aspect of meat extract has been presented so extensively in the numerous articles which from time to time have appeared in the literature that it is not considered necessary in this paper more than to refer to that phase of the question. As is well known, this product, now so generally used, owes its origin to Liebig, the chemist whose process for its preparation, as modified by Pettenkofer, has been in use in one of the large commercial houses ever since 1864.

In the method of preparation as originally described, muscle tissue alone was used for extraction, but in more recent years various influential factors, the foremost being the utilization of waste products, have caused many of the manufacturers to adapt the principles of the original process to the preparation of extracts from edible portions of the carcass other than true muscle tissue. Livers, spleens, hearts, cured-meat cook water,[1] roast-beef soak water, and bones to which more or less meat is adherent, are among the materials now employed, and the food analyst of to-day is confronted with many difficulties in his attempts to establish the identity of an extract under examination.

This investigation, therefore, was undertaken with the view of obtaining information regarding possible differences in composition of the various extracts that might be applied in formulating methods for their identification.

PREPARATION OF EXTRACTS

COMMERCIAL METHOD

Extracts of the various tissues and organs, such as chuck and plate (representing true muscle tissue), cured meat, bones (with and without adherent meat), hearts, livers, spleens, etc., were prepared, under the direct supervision of one of the authors, in the meat-extract department of one of the large commercial establishments. The method of preparation in each instance was that ordinarily used in the establishment, and

[1] Extracts were also prepared from the pickle in which the meats were cured, but the use of this material has been discontinued.

to all intents and purposes was practically the same in its essential features as that in use in the general commercial preparation of these articles. For the purpose of clarification "roast-beef soak water," "defibrinated blood," and "blood water," were added during the process of manufacture in all cases with the exception of the extracts prepared from cured meat. The comparatively large quantities employed of these agents necessarily influenced the composition of certain of the extracts, particularly those prepared from livers and spleens, and extracts of the various organs and tissues, therefore, were prepared in the laboratory, the method followed being nearly identical with the commercial process. Practically the only exception was the replacement of the materials commercially used in clarifying the extracts with those of a like composition, equally efficient, but derived from the specific tissue or organ under investigation. A detailed description of the laboratory process follows.

LABORATORY METHOD

The finely minced material from which the extract was prepared was placed in a large tin-lined box and iced water added until the minced meat was well covered. The box with its contents was then placed in the refrigerator where it was allowed to remain overnight, when the resulting "soak water" was drawn off and reserved for clarifying purposes. The partially extracted minced meat was then transferred to a large open kettle provided with a perforated steam coil, an equal weight of water added, and steam slowly applied, the temperature being gradually raised to 95° to 97° C., and the liquid kept in constant agitation by the entrance of the steam from the perforated pipe.

This extraction was continued for 45 minutes, after which the liquid was drawn off, cooled, and transferred to an evaporating kettle provided with a closed-coil steam pipe. The "soak water" obtained as above was then added, the whole brought to a boil, and the evaporation continued until the liquid was reduced to two-thirds of its original volume, the coagulable proteids which form a scum upon the surface of the liquid being removed from time to time. After this concentration the liquid was filtered and transferred to a vacuum kettle where it was evaporated under reduced pressure until the extract was of the desired consistence. This method yielded extracts identical in physical appearance and organoleptic properties with those obtained by the commercial process.

As it was also considered desirable to obtain data regarding possible differences in extracts prepared from cold and hot water extractions, the process described above was modified in the case of chuck and plate extracts prepared in the laboratory. Chuck and plate extract 29 was prepared by repeatedly exhausting the minced meat with large quantities of cold water and then concentrating the extract. Chuck and plate extract 30 was prepared by placing the minced meat in an equal

quantity of cold water, bringing the whole rapidly to a temperature of 95° to 97° C., where it was kept for 45 minutes, after which the liquor was drawn off and reduced by evaporation to the desired concentration.

It may be noted here that the two laboratory-prepared bone extracts, Nos. 27 and 28, were made by long-continued boiling of bones from which all meat had been removed.

List of extracts prepared

Commercially.	In the laboratory.
No. 10. Beef spleens.	No. 21. Beef spleens.
No. 11. Hog spleens.	No. 22. Beef spleens.
No. 12. Roast-beef soak water.	No. 23. Hog liver.
No. 13. Hog livers.	No. 24. Beef spleens.
No. 14. Bare beef bones.	No. 25. Hog liver.
No. 15. Regular bones.	No. 26. Beef hearts.
No. 16. Beef livers.	No. 27. Bones.
No. 17. Pickle.	No. 28. Bones.
No. 18. Beef hearts.	No. 29. Chuck and plate.
No. 19. Chuck and plate.	No. 30. Chuck and plate.
No. 20. Corned-beef cook liquor.	

QUANTITATIVE INVESTIGATION OF EXTRACTS

METHODS USED

In the analysis of the foregoing extracts the methods used were essentially those described by Street (*8*)[1] and, in brief, were as follows:

A 10 per cent solution of solid extract or a 20 per cent solution of liquid extract was used for the following determinations:

1. WATER.—The water representing the degree of concentration of the extract was determined by placing 20 cc. of the solution in a 100 cc. glass-stoppered weighing bottle containing 20 gm. of asbestos, and drying to constant weight in a vacuum of 30 inches at a temperature of 60° to 65° C.

2. ASH.—Ten cc. of the solution in a tared porcelain dish[2] were evaporated to dryness upon the steam bath, thoroughly carbonized at a low red heat, macerated with water, filtered, and the residue thoroughly washed and ignited. The filtrate was then added to the ignited residue in the dish, the whole evaporated to dryness upon the steam bath, ignited at a low red heat, and weighed.

3. SODIUM CHLORID.[3]—After weighing, the ash obtained was dissolved in water with the aid of a few drops of nitric acid, diluted to 100 cc., an aliquot taken, and chlorin determined by the Volhard method.[4]

[1] Reference is made by number (italic) to "Literature cited," p. 17.

[2] Porcelain was used instead of platinum in order that the possibility of volatilization of chlorin would be reduced to a minimum, as the ash was later utilized in the chlorin determination.

[3] Chlorin may be determined separately according to the method adopted by the Association of Official Agricultural Chemists. (2).

[4] Only a small portion of the chlorin of the ash of meat extracts is due to sodium chlorid, the greater portion being combined as chlorid of potassium (8). Allen (1) makes an allowance of 0.06 per cent sodium chlorid for every unit per cent of dry matter present, considering the excess as added salt.

4. TOTAL PHOSPHORIC ACID.—Five cc. of the solution were digested with 15 cc. each of sulphuric and nitric acids until colorless (nitric acid was added from time to time when necessary), 20 cc. of water were added, and the solution boiled in order to expel any oxids of nitrogen. It was then diluted with water, a slight excess of ammonium hydroxid added, after which it was rendered slightly acid with nitric acid, and phosphorus determined (*2*).

5. INORGANIC PHOSPHORIC ACID.—Ten cc. of the solution were diluted with from 20 to 30 cc. of water, boiled three minutes, two drops of acetic acid added, the boiling continued for a minute, cooled, and diluted to 100 cc. The solution was then filtered, a 50 cc. portion was made faintly alkaline with ammonium hydroxid, and the phosphoric acid precipitated in the usual manner with magnesia mixture. After standing for two hours or longer the precipitate was filtered off, washed with water containing 2.5 per cent of ammonia, and dissolved in dilute nitric acid. The phosphoric acid was then determined as in total phosphoric acid.

6. TOTAL NITROGEN.—Nitrogen was determined by the Gunning method, using 10 cc. of the solution.

7. SOLUBLE NITROGEN.—A portion of about 15 cc. of the solution was centrifuged until clear, the clear liquid poured off, and the nitrogen determined in a 10 cc. portion.

8. COAGULABLE NITROGEN.—Fifty cc. of the solution in a glass evaporating dish to which 50 cc. of water were added were evaporated on the steam bath to one-half volume; 0.5 cc. of a 10 per cent solution of acetic acid was added, heating was continued for 15 minutes, the coagulable albumen was filtered, washed, and nitrogen determined in the residue on the filter.

9. AMMONIA NITROGEN.—The ammonia nitrogen in these extracts was determined by the magnesium-oxid method, but the more recent and exact Folin method (*6*) is recommended.

10. NITROGEN PRECIPITATED BY ZINC SULPHATE.—Twenty-five cc. of the original solution were placed in a 50 cc. graduated flask, 1 cc. of a 50 per cent sulphuric-acid solution was added, with zinc sulphate enough to saturate the solution, after which the flask was filled to the mark with a saturated solution of zinc sulphate. After 18 hours it was filtered and the nitrogen determined by the Gunning method in 20 cc. of the filtrate, corresponding to 10 cc. of the original. The total nitrogen of the extract, less the sum of the coagulable, insoluble, and zinc-sulphate-filtrate nitrogen represents the nitrogen of the zinc-sulphate precipitate. A control determination of the nitrogen of the precipitate was also made.

11. NITROGEN PRECIPITATED BY TANNIC-ACID-SALT SOLUTION.—Twenty cc. of the original solution were placed in a 100-cc. graduated

flask, 50 cc. of a saturated sodium-chlorid solution were added, and the flask filled to the mark with a 24 per cent solution of tannic acid. After a thorough mixing it was placed in the ice box and allowed to stand overnight; any loss in volume due to contraction was corrected by the addition of the tannic-acid solution. On the following day it was filtered, the solution being kept in the ice box during filtration, and 50 cc. of the filtrate, corresponding to 10 cc. of the original, were transferred to a Kjeldahl flask and evaporated to dryness on the steam bath with the aid of a current of air. The nitrogen in the dried residue was determined by the Gunning method and control determinations made on the reagents used.

Nitrogen in the tannic-acid-salt precipitate was obtained by subtracting the sum of the tannic-acid-salt filtrate and the coagulable and insoluble nitrogen from the total nitrogen.

12. "MEAT-BASE" NITROGEN.—This was obtained by subtracting the sum of the coagulable, insoluble, ammonia, and tannic-acid-salt precipitate nitrogen from the total nitrogen.

13. NITROGEN DUE TO PEPTONE-LIKE BODIES.—This was found by deducting the proteose nitrogen obtained by precipitation with zinc sulphate from the total quantity of nitrogen precipitated by the tannic-acid-salt reagent.

14. NONNITROGENOUS ORGANIC MATTER.—This was determined by difference. From the ash-free total solids was deducted the sum of the products of the "meat-base" nitrogen × 3.12 and the nonmeat-base nitrogen × 6.25.

15. PURINS (*3*).—Three gm. of the sample were dissolved in 500 cc. of a 1 per cent solution of sulphuric acid and heated for four hours in an open dish on the steam bath. (At the end of this time about 75 cc. should remain.) It was then neutralized with caustic soda, with litmus paper as an indicator, transferred to a beaker, and 15 cc. of a 15 per cent solution of sodium bisulphite and 15 to 20 cc. of a 15 per cent solution of copper-sulphate solution were added. This was allowed to stand overnight, filtered, washed with dilute copper-sulphate solution, and the precipitate then washed with hot water from the paper into the original beaker. The contents of the beaker were brought to the boiling point and sodium sulphid added to precipitate all of the copper. It was then placed upon the steam bath for several minutes, made acid with acetic acid, and allowed to settle thoroughly, after which the precipitate was filtered off, washed with hot water, 10 cc. of 10 per cent hydrochloric acid added to the filtrate washings, and the solution evaporated to dryness on the steam bath. Ten cc. more of 10 per cent hydrochloric acid were added and digestion was continued until the bases in the residue were dissolved. It was then filtered, washed, the filtrate made alkaline with 25 cc. of concentrated ammonium hydroxid, 10 cc. of a 3 per cent

ammoniacal silver-nitrate solution added, allowed to stand overnight, filtered on the following morning, the residue on the paper washed until all traces of ammonia were removed, and its nitrogen content determined.

16. CREATININ.—The method of Folin as modified by Emmett and Grindley (5) was used. An aliquot free from coagulable and insoluble nitrogen and containing from 7 to 15 mgm. of creatinin was placed in a 500 cc. flask, 15 cc. of picric acid and 10 cc. of a 10 per cent solution of sodium hydrate added, allowed to stand for five minutes, being agitated several times in the interim, and then diluted to 500 cc. After mixing, a portion of the solution was poured into one tube of a Duboscq colorimeter and compared with *N/2* potassium-bichromate solution contained in the other tube, the scale of which was set at 8.0.

Creatinin was calculated by the following formula:

$$\left\{\frac{8.1}{\text{Reading}} \times \frac{\text{Volume}}{500}\right\} \times 10 = \text{milligrams of creatinin in the aliquot taken.}$$

17. CREATIN.—To 5 cc. of the extract in a 50 cc. graduated flask, 10 cc. of *N/1* hydrochloric acid and 5 cc. of water were added, and the solution heated in an autoclave at 135° C. for 30 minutes. It was then cooled, 10 cc. of *N/1* sodium hydroxid added and the solution made to volume with water. An aliquot was taken and creatinin determined as above, with 30 cc. of 1.2 per cent picric acid and 10 cc. of a 10 per cent solution of sodium hydroxid as suggested by Emmett and Grindley (5), the result so obtained representing the total creatinin—creatinin due to creatin and to preformed creatinin. The difference between the total creatinin and the preformed creatinin multiplied by 1.16 represents the creatin.

18. NITRATES.—To a few drops on a porcelain spot plate of a reagent containing 0.1 to 0.2 gm. of diphenylamin (4) in 100 cc. concentrated sulphuric acid were added a few drops of the extract solution. In the presence of nitrates a blue color developed. They were then quantitatively estimated by the Schlossing-Wagner method (9).

DISCUSSION OF QUANTITATIVE RESULTS

The results of the quantitative chemical examination of the extracts are presented in Table I and, calculated to a water-free basis, in Table II. In Table III differences in the forms of nitrogen are shown. The percentages of creatin and creatinin appear in Table IV together with the ratio between total nitrogen and the sum of the creatin and creatinin. The percentages in this table are also calculated on a water-free basis.

TABLE I.—*Results of chemical examination of meat extracts*

COMMERCIAL EXTRACTS

Extract No.	Water.	Total solids.	Ash.	Organic matter.	Nonnitrogenous organic matter.	Chlorin.			Phosphorus.			Acidity.		Nitrogen.											Preformed creatinin.	Creatin.	Nitrates.
						Chlorin.	Sodium chlorid (chlorin × 1.65).	Natural chlorids (total solids × 0.06).[a]	Total phosphorus pentoxid.	Inorganic phosphorus pentoxid.	Ratio of inorganic to total.	N/10 sodium hydroxid to 100 gm. of extract.		Total.	Insoluble.	Coagulable.	Ammonia.	Zinc sulphate precipitate.	Tannic-salt precipitate.	Peptone-like bodies.	Meat bases.								
												Litmus.	Phenolphthalein.								Total (by difference).	Creatin.	Preformed creatinin.	Purins.	Undetermined.			
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Cc.*	*Cc.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
10. Beef spleens	23. 29	76. 71	20. 39	56. 32	24. 96	3. 87	6. 45	4. 60	3. 10	2. 17	0. 700	[b] A	720	7. 66	0. 14	0. 00	0. 26	2. 30	3. 98	1. 68	3. 28	0. 21	0. 48	0. 69	1. 90	1. 31	0. 68	None.
11. Hog spleens	19. 46	80. 54	23. 01	57. 53	20. 55	3. 78	6. 30	4. 83	5. 17	3. 68	. 711	A	756	7. 56	. 00	. 06	. 29	2. 01	4. 05	2. 04	3. 10	. 18	. 44	. 80	1. 74	1. 21	. 59	None.
12. Roast-beef soak water	11. 70	88. 30	33. 64	54. 66	17. 49	7. 11	11. 85	5. 29	6. 40	5. 84	. 902	A	1, 121	7. 94	. 08	. 00	. 35	. 82	3. 53	2. 71	3. 98	. 25	2. 27	. 59	. 87	6. 13	. 79	None.
13. Hog livers	19. 00	81. 00	20. 78	60. 22	36. 47	4. 96	8. 28	4. 86	4. 67	2. 93	. 629	295	983	4. 85	. 01	. 05	. 09	. 91	2. 60	1. 69	2. 10	. 16	. 47	. 52	. 95	1. 29	. 50	None.
14. Bare beef bones	16. 10	83. 90	29. 55	54. 35	17. 06	5. 55	9. 25	5. 03	5. 53	4. 98	. 900	A	942	7. 95	. 10	. 00	. 23	1. 13	3. 98	2. 85	3. 64	. 37	1. 92	. 58	. 67	5. 18	1. 16	None.
15. Regular bone	17. 00	83. 00	28. 62	54. 28	19. 05	5. 40	9. 00	4. 98	5. 14	4. 56	. 904	A	857	7. 96	. 16	. 02	. 23	1. 20	3. 55	2. 35	4. 00	. 42	2. 02	. 57	. 99	5. 46	1. 32	None.
16. Beef livers	18. 18	81. 82	21. 76	60. 06	39. 98	4. 86	8. 11	4. 91	5. 25	3. 31	. 630	695	1, 192	5. 40	. 03	. 06	. 09	1. 70	3. 02	1. 32	2. 20	. 23	. 46	. 51	1. 00	1. 26	. 73	None.
17. Pickle	11. 10	88. 90	27. 50	61. 40	31. 95	5. 62	9. 37	5. 33	1. 52	1. 39	. 914	509	1, 437	6. 66	. 00	. 05	. 27	. 76	2. 45	1. 69	3. 89	. 07	1. 14	. 38	2. 30	3. 09	. 25	14. 45
18. Beef hearts	21. 30	78. 70	24. 48	54. 22	22. 00	4. 57	7. 63	4. 72	5. 49	4. 64	. 845	A	1, 046	7. 10	. 06	. 00	. 40	1. 23	2. 82	2. 56	3. 88	. 32	1. 34	. 65	1. 48	3. 65	1. 00	None.
19. Chuck and plate	19. 80	80. 20	23. 20	57. 00	19. 43	3. 23	5. 39	4. 81	6. 16	5. 28	. 844	493	1, 073	8. 09	. 01	. 06	. 30	1. 44	3. 57	2. 13	4. 15	. 51	1. 69	. 57	1. 38	4. 57	1. 61	None.
20. Corned-beef cook liquor	17. 00	83. 00	23. 78	59. 22	22. 43	5. 27	8. 79	4. 98	1. 87	1. 42	. 760	197	809	7. 66	. 08	. 06	. 50	1. 32	3. 16	1. 84	3. 86	. 36	1. 24	. 58	1. 68	3. 34	1. 13	8. 70

LABORATORY EXTRACTS

21. Beef spleens	24. 78	75. 22	19. 22	57. 00	17. 36	2. 58	4. 33	4. 51	5. 36	.....	.....	A	815	7. 51	0. 10	0. 14	0. 74	1. 68	4. 21	2. 53	2. 32	0. 00	0. 06	0. 91	1. 35	0. 18	0. 01	None.
22. Beef spleens	28. 09	71. 91	16. 57	55. 34	16. 71	1. 91	3. 19	4. 31	5. 22	4. 19	0. 802	A	825	7. 75	. 17	. 00	. 81	1. 74	3. 64	1. 90	3. 13	. 00	. 06	1. 08	1. 99	. 17	. 01	None.
23. Hog liver	19. 75	80. 25	21. 36	58. 89	24. 67	2. 50	4. 17	4. 80	7. 75	4. 45	. 575	98	790	6. 53	. 00	. 06	. 59	1. 58	3. 84	2. 26	2. 04	. 03	. 08	. 25	1. 68	. 22	. 03	None.
24. Beef spleens	30. 08	69. 92	20. 29	49. 63	16. 56	3. 08	5. 14	4. 19	5. 41	3. 64	. 672	A	651	6. 36	. 00	. 40	. 30	1. 33	3. 53	2. 20	2. 13	. 00	. 08	. 43	1. 62	. 22	. 02	None.
25. Hog liver	19. 36	80. 64	15. 98	64. 66	39. 55	2. 15	3. 59	4. 80	10. 59	3. 35	. 317	251	929	5. 26	. 04	. 07	. 24	. 60	2. 75	2. 15	2. 16	. 01	. 07	. 29	1. 79	. 19	. 03	None.
26. Beef hearts	19. 91	80. 09	23. 24	56. 85	25. 05	2. 66	4. 44	4. 80	6. 52	5. 57	. 854	279	1, 058	7. 04	. 12	. 00	. 83	. 78	2. 15	1. 37	3. 93	. 47	1. 65	. 57	1. 25	4. 16	1. 28	None.
27. Bones [c]	57. 35	42. 65	8. 09	33. 56	13. 21	1. 78	2. 97	2. 52	. 71	. 03	. 887	59	59	3. 52	1. 18	1. 46	. 09	. 54	1. 26	. 72	. 53	. 00	. 22	. 05	. 26	. 59	. 00	None.
28. Bones [c]	44. 97	55. 03	18. 86	36. 17	10. 25	5. 50	9. 18	3. 80	. 81	. 46	. 567	26	.....	4. 64	. 00	. 09	. 18	2. 58	3. 57	. 99	. 90	. 01	. 19	.....	. 70	. 53	. 04	None.
29. Chuck and plate [d]	11. 63	88. 37	27. 13	61. 24	23. 72	2. 52	4. 20	.....	6. 78	.....	.....	889	1, 581	8. 57	.....	. 03	. 70	.....	2. 39	.....	5. 45	. 11	2. 41	. 68	2. 25	6. 48	. 37	None.
30. Chuck and plate [e]	17. 21	82. 79	23. 48	59. 31	12. 14	2. 03	3. 39	.....	6. 11	4. 82	. 788	348	946	9. 66	.....	. 05	. 65	2. 09	4. 73	2. 64	4. 23	. 91	. 92	. 70	1. 71	2. 46	2. 84	None.

[a] See Allen (1, p. 307).
[b] Amphoteric.
[c] These extracts gave a very cloudy solution, muddy-like, and had an offensive soapy odor.
[d] Cold-water extract; extracted four times with cold water, then boiled and concentrated.
[e] Hot-water extract; placed in tcold water and immediately brought to a boil.

TABLE II.—*Results of chemical examination of meat extracts (water-free basis)*

COMMERCIAL EXTRACTS.

Extract No.	Ash.	Organic matter.	Nonnitrogenous organic matter.	Chlorin.		Phosphorus.			Nitrogen.													Creatinin.	Creatin.
				Chlorin.	Sodium chlorid (Chlorin×1.65).	Total phosphorus pentoxid.	Inorganic phosphorus pentoxid.	Ratio of inorganic to total.	Total.	Insoluble.	Coagulable.	Ammonia.	Zinc-sulphate precipitate.	Tannic-salt precipitate.	Peptone-like bodies.	Meat bases.							
																Total (by difference).	Creatin.	Creatinin.	Purins.	Undetermined.		
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
20. Beef spleens	26.59	73.41	24.07	5.04	8.41	4.04	2.83	0.700	9.98	0.18	0.00	0.34	3.00	5.17	2.17	4.16	0.27	0.62	0.88	2.47	1.70	0.88
21. Hog spleens	28.58	71.42	25.23	4.70	7.83	6.42	4.57	.711	9.38	.00	.07	.36	2.49	4.92	2.43	3.91	.22	.54	.99	2.14	1.50	.73
22. Roast-beef soak water	38.10	61.90	19.70	8.05	13.42	7.25	6.62	.902	8.99	.09	.00	.39	.92	3.96	3.04	4.49	.28	2.58	.67	.98	6.94	.89
23. Hog livers	25.65	74.35	44.96	6.12	10.22	5.76	3.62	.629	6.00	.03	.05	.11	1.12	3.20	2.08	2.59	.20	.58	.65	1.16	1.59	.61
24. Bare beef bones	35.20	64.80	19.17	6.60	11.02	6.55	5.94	.900	9.47	.12	.00	.28	1.34	4.73	3.39	4.33	.44	2.28	.69	.79	6.18	1.38
25. Regular bones	34.48	65.52	31.62	6.50	10.84	6.07	5.49	.904	9.59	.20	.00	.29	1.44	4.27	2.83	4.80	.50	2.42	.68	1.18	6.60	1.59
26. Beef livers	26.58	73.42	40.62	6.01	10.03	6.42	4.05	.630	6.59	.03	.07	.11	2.07	3.68	1.61	2.68	.28	.56	.62	1.22	1.54	.89
17. Pickle	30.92	69.08	29.30	6.32	10.54	1.76	1.56	.914	7.60	.....	.02	.30	.86	2.64	1.78	4.35	.08	1.28	.42	2.57	3.48	.28
18. Beef hearts	31.10	68.90	29.74	5.82	9.70	6.98	5.90	.845	9.02	.08	.00	.49	1.55	3.61	2.06	4.84	.40	1.70	.77	1.89	4.63	1.26
19. Chuck and plate	28.90	71.10	24.20	4.03	6.72	7.66	6.46	.844	10.08	.00	.08	.37	1.79	4.45	2.66	5.14	.63	2.11	.70	1.71	5.69	2.00
20. Corn-beef cook liquor	28.65	71.35	28.21	6.35	10.59	2.25	1.71	.760	9.23	.10	.07	.60	1.59	3.79	2.20	4.63	.73	1.48	.70	2.01	4.02	1.36
LABORATORY EXTRACTS																						
21. Beef spleens	25.56	74.44	24.79	3.45	5.75	7.12	.....	.....	9.98	0.13	0.18	0.98	2.37	5.60	3.23	3.08	0.00	0.07	1.21	1.80	0.23	0.01
22. Beef spleens	23.03	76.97	23.51	3.25	5.43	7.25	5.82	0.803	10.77	.23	.00	1.13	2.41	4.82	2.41	4.35	.00	.08	1.50	2.77	.23	.01
23. Hog livers	26.51	73.49	29.54	3.11	5.19	9.65	5.54	.575	8.14	.00	07	.73	1.97	4.75	2.78	2.53	.00	.10	.31	2.11	.39	.04
24. Beef spleens	29.01	70.99	24.11	4.47	7.45	7.73	5.19	.679	9.07	.00	.56	.42	1.91	5.04	3.13	3.35	.00	.11	.61	2.63	.31	.03
25. Hog livers	19.81	80.19	48.79	2.67	4.45	13.13	4.15	.316	6.52	.05	.09	.29	.61	3.41	2.80	2.68	.00	.08	.36	2.23	.24	.04
26. Beef hearts	28.98	72.02	32.57	3.31	5.53	8.13	6.93	.854	8.77	.14	.00	1.03	.98	2.69	1.71	4.91	.53	.05	.71	1.62	6.64	1.60
27. Bones	28.90	78.76	31.06	4.18	6.97	1.76	1.48	.887	8.26	.43	3.42	.18	1.26	1.66	1.82	1.24	.00	.51	.11	.61	1.12	.00
28. Bones	33.98	65.19	18.00	10.43	15.73	1.46	.83	.561	8.36	.00	.16	.32	4.81	6.44	1.63	1.62	.....	.34	.....	1.26	1.02	.07
29. Chuck and plate	30.68	69.32	28.42	2.85	4.75	7.67	.....	.....	9.69	.....	.04	.79	.....	2.70	.....	6.16	.13	2.73	.77	2.54	7.32	.41
30. Chuck and plate	28.36	71.64	24.70	2.44	4.09	7.38	5.82	.788	11.67	.....	.06	.78	2.52	5.71	3.19	5.11	1.27	1.10	.84	2.06	2.97	3.43

TABLE III.—*Distribution of nitrogen in meat extracts*

Extract No.	Method of preparation.	Non-nitrogenous matter.	Total nitrogen.	Total nitrogen in—		
				Zinc-sulphate precipitate.	Tannic-salt precipitate.	"Meat base."
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
19. Chuck and plate	Commercial	24. 20	10. 08	17. 75	44. 13	50. 98
29. Chuck and plate	Laboratory	28. 02	9. 69	.....	27. 86	63. 56
30. Chuck and plate	do	14. 70	11. 67	21. 57	48. 87	43. 74
12. Roast-beef soak water	Commercial	19. 70	8. 99	10. 34	44. 05	49. 94
20. Corn-beef cook liquor	do	28. 21	9. 23	17. 21	41. 04	50. 13
14. Beef bones	do	19. 17	9. 47	13. 58	47. 96	43. 90
15. Beef bones	do	21. 62	9. 59	15. 00	44. 49	50. 01
17. Pickle	do	29. 30	7. 60	11. 31	34. 71	57. 20
18. Beef hearts	do	29. 74	9. 02	17. 17	39. 99	53. 62
26. Beef hearts	Laboratory	32. 57	8. 77	11. 17	30. 76	55. 97
10. Beef spleens	Commercial	24. 07	9. 98	30. 07	51. 81	41. 68
11. Hog spleens	do	25. 23	9. 38	26. 53	52. 45	41. 68
21. Hog spleens	Laboratory	24. 79	9. 98	23. 74	56. 10	30. 87
22. Hog spleens	do	23. 51	10. 77	22. 36	44. 63	40. 37
24. Hog spleens	do	24. 11	9. 07	21. 14	55. 54	36. 91
13. Hog liver	Commercial	44. 96	6. 00	18. 66	53. 33	43. 16
16. Beef liver	do	40. 63	6. 42	32. 23	57. 29	41. 72
23. Hog liver	Laboratory	30. 54	8. 14	24. 19	58. 37	31. 06
25. Hog liver	do	48. 79	6. 52	9. 35	52. 30	41. 10
Averages:						
Chuck and plate, bones, liquors		22. 23	9. 81	15. 90	42. 63	50. 32
Hearts		31. 65	8. 89	14. 17	35. 33	54. 79
Spleens		24. 34	9. 81	24. 76	52. 10	38. 30
Livers		41. 22	6. 77	21. 10	55. 32	39. 26

In consulting these tables it will be noted that the percentage quantities of certain constituents show marked and characteristic differences, depending upon the nature of the extract. The most striking variations are the figures representing total nitrogen, "meat-base" nitrogen, creatinin, and nonnitrogenous organic matter. Differences in the amounts of the other constituents, with the exception of the ratio of total phosphorus to inorganic phosphorus, are not considered sufficiently marked to justify their being used, and attention is directed to the following results:

1. TOTAL NITROGEN.—This was found to be very low in liver extracts, as compared with other extracts. The percentage of total nitrogen in one of the liver extracts (No. 23) is much higher than that of the remaining three, but is, nevertheless, lower than that of any of the other extracts with the exception of the pickle extract. Chuck and plate extracts contain the largest quantity of nitrogen, with spleen extracts next. The other extracts vary between 9 and 10 per cent total nitrogen with the exception of the pickle extract, which is very low (7.60 per cent).

TABLE IV.—*Distribution of creatin and creatinin (water-free basis)*

Extract No.	Method of preparation.	Total nitrogen.	Creatin.	Creatinin.	Total creatin plus creatinin.	Ratio of creatin plus creatinin to nitrogen.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
19. Chuck and plate	Commercial	10.08	2.00	5.69	7.69	0.763
29. Chuck and plate	Laboratory	9.69	.41	7.32	7.73	.797
30. Chuck and plate	do	11.67	3.43	2.97	6.40	.463
12. Roast-beef soak water	Commercial	8.99	.89	6.94	7.83	.870
20. Corn-beef cook liquor	do	9.23	1.36	4.02	5.38	.583
14. Beef bones	do	9.47	1.38	6.18	7.56	.798
15. Beef bones	do	9.59	1.59	6.60	8.19	.854
17. Pickle	do	7.60	.28	3.48	3.76	.493
18. Beef hearts	do	9.02	1.26	4.63	5.89	.653
26. Beef hearts	Laboratory	8.77	1.60	6.64	8.24	.939
10. Beef spleens	Commercial	9.98	.88	1.70	2.58	.258
11. Hog spleens	do	9.38	.73	1.50	2.23	.237
21. Hog spleens	Laboratory	9.98	.01	.23	.24	.024
22. Hog spleens	do	10.77	.01	.23	.24	.022
24. Hog spleens	do	9.07	.03	.31	.34	.037
13. Hog liver	Commercial	6.00	.61	1.59	2.20	.366
16. Beef liver	do	6.42	.89	1.54	2.43	.368
23. Hog liver	Laboratory	8.14	.04	.39	.43	.053
25. Hog liver	do	6.52	.04	.24	.28	.043
Averages:						
Chuck and plate, bones, liquors		9.81	.....	.....	7.25	.732
Hearts		8.89	.....	.....	7.06	.796
Commercial spleens		9.68	.....	.....	2.40	.248
Laboratory spleens		9.94	.....	.....	.27	.031
Commercial livers		6.21	.....	.....	2.31	.367
Laboratory livers		7.33	.....	.....	.36	.048

2. NONNITROGENOUS ORGANIC MATTER.—As will be noted in Table III, liver extracts, as compared with the other extracts, are extraordinarily high in nonnitrogenous organic matter, containing, with the exception of extract 23, more than 40 per cent. Heart extracts (containing 30 per cent) more nearly resemble liver extracts.

3. "MEAT-BASE" NITROGEN.—The determination of total nitrogen and of "meat-base" nitrogen enables one with a fair degree of certainty to differentiate liver extracts and spleen extracts from each other and from other extracts as well. While liver extracts and spleen extracts differ from other extracts in showing a low percentage of "meat-base" nitrogen, they differ from each other in that the spleen extracts show a high total nitrogen, whereas the total nitrogen of liver extracts is low.

In liver extracts the "meat-base" nitrogen constitutes only about 40 per cent of the total nitrogen, while in other extracts, with the exception of spleen extracts, the figure is nearer 50 per cent. Particular attention is called to liver extract 23, which contains 8.14 per cent total nitrogen and pickle extract 17, containing 7.60 per cent total nitrogen. Although the pickle extract contains much less total nitrogen than the liver extract, a much larger proportion of this nitrogen (57.23 per cent) is "meat-base"

nitrogen. In the liver extract the "meat-base" nitrogen constitutes only 31.08 per cent of the total nitrogen.

Chuck and plate extract 30 is much lower in "meat-base" nitrogen than the other chuck and plate extracts. This is exceptional and is undoubtedly due to the laboratory process used in its preparation, the extraction having been made entirely with hot water. (See p. 2.)

4. PROTEOSE NITROGEN (zinc-sulphate precipitate, Table III).—Although the quantity of the proteose nitrogen varies from 9.35 to 32.23 per cent of the total nitrogen, the amounts in any one kind of extract are not sufficiently constant to render the figure of any value in the identification of extracts. On the whole, however, liver and spleen extracts are somewhat higher in that constituent than other extracts. This factor is probably influenced more by the process used in the preparation of the extract than by the material from which the extract is made.

5. CREATIN AND CREATININ.—It is in the total creatinin content of the various extracts that the greatest and most uniform differences occur. The sum of the quantities of creatin and creatinin, together with the ratio between this total and the total nitrogen of the extracts, is shown in Table IV. It appears from these results that a determination of the total creatinin will suffice in any case to classify an extract, if pure, as a liver or spleen extract, on the one hand, or as a true meat extract on the other.[1]

It will be noticed at once that the liver and spleen extracts prepared under commercial conditions contain about 10 times as much total creatinin as the laboratory extracts, attributable to the creatinin of the roast-beef soak water, defibrinated blood, and blood water used in clarifying these extracts. However, even though these commercially prepared liver and spleen extracts are relatively high in creatinin, they are, nevertheless, much lower than any of the other extracts. The greatest quantity of creatinin found in any of the liver and spleen extracts is 2.58 per cent and the highest total creatinin—total nitrogen ratio—0.37, while the smallest amount of creatinin in the other extracts (except the pickle extract) is 5.38 per cent and the lowest ratio 0.46. From these results it appears that all extracts of fresh flesh, with the exception of extracts of liver and spleen, contain more than 5 per cent of total creatinin.

6. PHOSPHORUS.—Rather marked differences occur in the amounts of phosphorus found in the extracts as well as in the relation existing between the inorganic and total phosphorus present.

The extracts of pickle and of corned-beef cook liquor contain very little phosphorus, about 2 per cent; none of the other extracts contain less than 5 per cent with the exception of spleen extract 10. The laboratory liver extracts are noticeably high in phosphorus.

[1] Extracts from bones as made commercially will show a relatively high total creatinin. This is due to the fact that the commercial bone extracts are essentially meat extracts, most of the extractives in them being derived from the adherent meat and the clarifying agents which are used, and not from the bones themselves.

Differences which are highly characteristic are found in the relation of total and inorganic phosphorus, the ratio of inorganic phosphorus pentoxid to total phosphorus pentoxid being much lower for liver than for other extracts, the next higher being that of spleens. Grouping these ratios, livers have a ratio lower than 0.65; spleens a ratio near 0.7; hearts, chuck, and plate, and corned-beef cook liquor run above 0.75 and nearer 0.8; and the ratio in the remaining extracts is 0.90 or higher.

SUMMARY OF QUANTITATIVE DIFFERENCES

Liver extracts are low both in total nitrogen and "meat-base" nitrogen; have a low inorganic phosphorus to total phosphorus ratio, are very low in total creatinin, and as a rule are very high in nonnitrogenous organic matter.

Spleen extracts are high in total nitrogen, low in "meat-base" nitrogen, very low in creatinin, and lower than other extracts, liver excepted, in the inorganic-phosphorus to total-phosphorus ratio.

Heart extracts are low in total nitrogen as compared with chuck and plate extracts, but much higher than liver. They contain considerable nonnitrogenous organic matter, being next to liver extracts in this respect. Heart extracts differ from liver and spleen extracts in total creatinin and in "meat-base" nitrogen, the latter comprising at least 50 per cent of the total nitrogen in heart extracts.

Pickle and cured-meat extracts are readily identified by the presence of nitrates, which are always present in such extracts. The quantity of total phosphorus present in such extracts is very small. In other respects cured-meat extracts are found to resemble true-meat extracts. Pickle extracts contain rather less creatinin than true-meat extracts.

Chuck and plate extracts run high in total nitrogen, "meat-base" nitrogen, and total creatinin and have a high inorganic-phosphorus to total-phosphorus ratio.

The bone extracts prepared commercially [1] and the extract prepared from roast-beef soak water resemble chuck and plate extract.

PHYSICAL CHARACTERISTICS OF EXTRACTS

In addition to the chemical differences which have been discussed above marked physical characteristics of the extracts exist which in many cases are so decided that workmen engaged in their manufacture become very expert in identifying meat extracts solely by their physical appearance. The properties upon which their judgment is based are color, texture, and "shortness," an extract being termed "short" when it quickly and easily breaks upon testing its elasticity.

[1] The bone extracts prepared in the laboratory have not been discussed, as they do not in the least resemble commercial bone extracts, and are included in Tables I and II merely as a matter of general interest.

Liver extracts are very dark brown, almost black, in color, are very gummy—that is, they are not "short," and their solution in water is dark red, with a trace of fluorescence.

Spleen extracts are light-chocolate to light yellow-brown in color have a smooth texture, and are very "short."

Bone extracts closely resemble spleen extracts.

Other extracts, including heart extract, are darker than spleen extract, but not so dark as liver extract. They are usually very "short," and their solutions are dark, but are not fluorescent.

QUALITATIVE INVESTIGATION OF EXTRACTS

In addition to the quantitative differences in extracts qualitative differences have been noted and, based on these differences, qualitative tests have been devised for the identification of liver and spleen extracts either when pure or in the absence of any considerable proportions of true-meat extracts. In mixtures in which liver or spleen extracts are present in only small amounts the tests are not entirely dependable, although in such instances they are as reliable as any other known method.

ACETIC-ACID TEST

A qualitative test for the identification of spleen extracts was suggested by Robert M. Chapin, of the Biochemic Division, who noticed that the addition of an excess of acetic acid to a spleen extract resulted in the formation of an abundant precipitate. Confirmation of this observation was found in the literature, Hammarsten (7) stating that spleens are characterized by a peculiar protein which is soluble in boiling water, but which is precipitated by an excess of acetic acid.

Acting upon this information, the writers tested all the commercially prepared spleen extracts (the laboratory-prepared extracts having been exhausted in the quantitative investigation) with acetic acid in the manner described below.

About 30 cc. of a 10 per cent solution of the extract under examination are boiled, filtered, the filtrate cooled, and an equal quantity of a 10 per cent solution of acetic acid added.

Extract No.	Effect of acetic acid.
10. Beef spleens	A dense, white precipitate.
11. Hog spleens	A dense, white precipitate.
12. Roast-beef soak water	No effect.
13. Hog livers	A slight, dark precipitate.
14. Bare beef bones	No effect.
15. Regular bones	No effect.
16. Beef livers	A slight, dark precipitate.
18. Beef hearts	No effect.
19. Chuck and plate	No effect.
20. Corned-beef cook liquor	A very slight yellowish precipitate.

With spleen extracts only was a decided precipitate obtained. The precipitate was very bulky and yellowish white in color, easily distinguishable from both the slight, dark precipitate yielded by the liver extracts and the slight precipitate obtained with the corned-beef cook liquor.

Mixtures of varying amounts of hog spleens (No. 11) and chuck and plate extract (No. 19) were prepared and tested with acetic acid in the manner shown above, in order to determine the delicacy of the reaction. A precipitate was obtained in mixtures containing 5 per cent of spleen extract, but the reaction was faint, and it was not until the mixed extract contained a quantity approximating 20 per cent that a decided reaction was obtained.

Since the above tests were made an extract has been received and examined under the meat-inspection regulations which gave a positive reaction with the acetic-acid test, although not resembling spleen extracts in other respects.

An investigation by the field service of the Meat Inspection Division disclosed that this extract was prepared from bones through long-continued extraction with boiling water. Inasmuch as bone extract previously examined had not given the reaction, a laboratory investigation of this unusual feature was made, three bone extracts being prepared by extraction with boiling water for at least three hours. Upon testing the finished products the reaction, which heretofore had been limited to spleen extracts, was obtained. The precipitate from both the spleen and these bone extracts appeared to be a mucin. From the standpoint of the food analyst the value of the test, however, is not lessened, as the differentiation of bone extracts from other extracts, including spleen, is readily made, which may be noted by comparing the analyses given in Table V with those of other extracts previously tabulated (see Table II).

TABLE V.—*Analyses of bone extracts*

Constituent.	Extract 1.	Extract 2.	Extract 3.
	Per cent.	*Per cent.*	*Per cent.*
Total solids	57.54	62.90	63.53
Ash	3.42	13.80	14.81
Sodium chlorid	.81	7.58	7.32
Phosphorus pentoxid (total)	.29	.49	.59
Nitrogen	8.54	7.90	10.40
Tannic-acid salt filtrate nitrogen	.35	1.46	1.37
Zinc-sulphate filtrate nitrogen	3.23	2.21	3.60
Total creatinin	None.	None.	None.
Acetic-acid test	Positive.	Positive.	Positive.
Molisch test	Negative.	Negative.	Negative.

While not applying to the real purpose of this paper, it may be of interest to note that an extract of bone marrow failed to afford a precipitate with acetic acid.

MOLISCH TEST

By determining the total creatinin content of an extract, the water content being known, it can be definitely classified as either a true-meat extract or as an extract of liver or spleen, and further, an extract identified as having been prepared from either livers or spleens may be further classified as either a spleen or a liver extract, depending upon the reaction in the acetic-acid test. While the identity of a liver extract may thus be established, qualitative tests confirmatory of the conclusions arrived at through the quantitative examination were applied. A reaction based on the occurrence in liver extracts of comparatively large amounts of carbohydrates was suggested and the Molisch test was employed.

METHOD OF USING MOLISCH TEST.—One cc. of a 10 per cent solution of solid or of a 20 per cent solution of fluid extract was placed in a graduated glass-stoppered cylinder of 25 cc. capacity, 9 cc. of concentrated sulphuric acid were allowed to flow gently down the sides of the cylinder, and 6 to 10 drops of a 20 per cent alcoholic solution of alphanaphthol were then added. The stopper was inserted, and the contents of the cylinder were thoroughly mixed. In the presence of carbohydrates a persistent and intense reddish-purple to deep-violet color developed immediately.

In the initial tests the color of the mixture in the cylinder was noted one minute after shaking, and the contents of the cylinder were then poured into 200 cc. of water, the color again being observed. It was later found that a more satisfactory color test was obtained when the mixture in the cylinder was allowed to stand overnight and observed directly.

Extracts of known origin were tested with the results given in Table VI.

TABLE VI.—*Results of Molisch test of meat extracts of known origin*

Extract.	Method of preparation.	Color on shaking.[a]	Color in water.[a]	Color after standing.[a]
Roast-beef soak water	Commercial	No color	No color	No color.
Beef bones	do	do	do	Do.
Beef chuck	do	do	do	Do.
Beef spleens	do	Very faint purple.	Very faint purple.	Do.
Hog spleens	do	do	do	Do.
Beef liver	do	Strong	Strong	Strong.
Do	do	Pronounced	Pronounced	Pronounced.
Beef chuck	Laboratory	Very faint	Very faint	No color.
Hog liver	Commercial	Pronounced	Pronounced	Pronounced.
Beef melts (spleens)	Laboratory	Very faint	Very faint	Very faint.
Beef liver	do	Pronounced	Pronounced	Pronounced.
Do	do	do	do	Do.
Do	do	do	do	Do.

[a] In cases marked "no color" there was no trace of the characteristic purple-red color, but there was usually a greenish-brown color.

Mixtures of liver extracts with chuck and plate extracts in varying proportions were prepared for the purpose of determining the delicacy of the reaction, and it was found that the characteristic color was recognizable in an extract containing 20 per cent of liver extract; smaller quantities gave a purple color, but it was not sufficiently distinct to be of value.

In all cases where a positive reaction is obtained and in which the other factors, such as creatinin, nitrogen, etc., indicate the absence of liver extract, the sample should be examined for starch and cane sugar.

It may be stated that, aside from its value in indicating the presence of liver extract, the Molisch test is necessary in a routine examination for the rapid detection of carbohydrates which may have been added to meat extracts.[1] In the analyses of some hundreds of extracts a true-meat extract has never yielded a positive reaction with this test, and whenever a positive reaction is obtained in an extract which can be shown to contain no liver extract it is due to added carbohydrate. In such instances the test should be supplemented with a more complete examination to identify the carbohydrate thus indicated.

COPPER TEST

During the course of the investigation it was also noted that the ash of liver extracts in every instance exhibited a more or less pronounced greenish color, which was not observed in the ash of any of the large number of other kinds of extracts examined. As the presence of copper in livers has been demonstrated, it having been found even in the liver of the fetus, the presence of copper in the ash of extracts other than those prepared from livers should be considered. As practically all the commercial extracts prepared from other tissues and organs underwent the same course of preparation, and no copper was thus indicated in the ash of any of the resulting products, it would seem that this test would apply alone to the ash of extracts obtained from livers. If, however, copper utensils are used in the preparation of an extract its presence in limited amounts could probably be readily demonstrated by chemical methods, but a greenish-tinted ash should always lead one to suspect the presence of liver extract.

Procedure suggested in the identification of an extract:

1. Total solids.
2. Ash.
3. Sodium chlorid.
4. Total phosphoric pentoxid.
5. Inorganic phosphoric pentoxid.
6. Total nitrogen.
7. "Meat-base" nitrogen.
8. Preformed creatinin.
9. Creatin.
10. Molisch test.
11. Acetic-acid test.
12. Test for starch and sugar if a positive Molisch test is given.
13. Test for nitrates.
14. Test of ash for copper.

[1] As an illustration of the value of this test, regardless of its value in detecting the presence of liver, several extracts have been examined which conformed in every respect to pure-meat extracts with the exception that they gave a positive Molisch test. Upon investigation the presence of sucrose was demonstrated.

The quantities of insoluble, coagulable, and ammonia nitrogen are so small in all ordinary extracts that they are determined only in case they are indicated in extraordinary amounts. Should an extract show an unusually high nitrogen content a study of the various forms of nitrogen present is essential.

After an examination of an extract as suggested its accurate classification as a true-meat extract, as a cured-meat extract, as a compound extract, or as an extract of liver or spleen is possible, and at the same time the addition of foreign material, such as sugar, starch, or salt, will be established.

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QUANTITY AND COMPOSITION OF EWES' MILK: ITS RELATION TO THE GROWTH OF LAMBS

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INTRODUCTION

During the progress of an investigation upon different breeds of sheep at the Idaho Agricultural Experiment Station observations were made upon the rate of growth of lambs from five breeds of ewes that are commonly found in this section of the country. The results indicated that lambs from some of the breeds studied made a decided gain over others in the same period of time. Inasmuch as the sheep industry is of such economic importance to the Nation, the rate of growth of the lambs assumes more than ordinary interest, and an effort is being made to ascertain the relation of the quantity and composition of ewes' milk of each breed to the growth of the lambs.

FACTORS ESSENTIAL IN GROWTH

The chief factors in growth are inherited capacity and a sufficient quantity of nutritious food. The second factor only will be studied, since without sufficient food inherited capacity for growth will be more or less inhibited.

Growth depends upon nutritious foods, and recent investigators have demonstrated that these foods must contain a sufficient quantity of inorganic salts, certain amino acids, lipoids, fats or oils of a peculiar nature, and vitamines. The absence of any of these substances is detrimental to growth. It is obvious that a well-balanced food is essential, and in milk we have the highest type of such food. The fact, however, remains that milk from ewes of different breeds has been found to vary in quantity and composition, and this may account to some extent for differences in growth.

REVIEW OF LITERATURE

As early as 1850 data were collected giving the analysis of ewes' milk. Since that time many investigators [2] have contributed to our knowledge of the composition of ewes' milk. Their results, however, have dealt mainly with the high-milk-producing ewes of foreign countries, where

[1] The authors wish to acknowledge with thanks the careful work of the following men whose assistance made it possible to carry on this work: To Messrs. Grover D. Turnbow, R. R. Groninger, and Ronald Wood credit is due for the chemical analyses; to Messrs. O. W. Johnson, C. H. Ficke, and W. H. Booth (killed in service, France) for the careful determination on yield of milk and growth of lambs.

[2] KÖNIG, J. CHEMIE DER MENSCHLICHEN NAHRUNGS- UND GENUSSMITTEL. Aufl. 4, Bd. 1, p. 265-271. Berlin, 1903.

yield of milk and the butter-fat content was of greatest consequence. These results are of little benefit in the solution of this problem, other than to show the great variation that occurs between different breeds and within the breeds. This problem will include only such breeds as are common to this section of the country. Among the above-mentioned investigations, only two give figures upon breeds that will be included in this work. Filhol and Joly [1] give figures upon the Southdown, and Hucho [2] upon the Hampshire breed. These results serve only to verify the results of other investigators, and show the variation between breeds.

Fuller and Kleinheinz,[3] of the Wisconsin Station, made a study of the yield, fat, and total solids of the milk of five breeds of sheep; the Oxford, Southdown, Dorset, Shropshire, Merino, and the Montana grade. They included two ewes of each breed in their study, and took the average of the two results as the average of the breed. In determining the milk yield, the lamb was weighed before and after sucking the mother ewe. This was repeated at frequent intervals during a 48-hour period, from which the yield of milk for 24 hours was calculated. They observed that, when the ewes were milked by hand, only about one-half the quantity of milk was obtained as when the first method was used. The results on the two ewes of each breed show a wide variation in milk yield and percentage of fat between the breeds.

Ritzman,[4] of the New Hampshire Station, in a recent publication has made a valuable contribution to the present knowledge of ewes' milk. His work dealt especially with the fat content and its relation to growth of lambs. A summary of his results on the fat content of 6 distinct breeds and 11 crossbreeds over a considerable period of years showed a great variation in the percentage of fat. The outstanding feature was that not only did breeds differ in fat content of milk, but individual ewes within the breed differed greatly. Moreover, these individual ewes showed marked differences in fat percentage at different lactation periods. This fact was observed by the writers during a preliminary investigation of ewes' milk carried on a year previous to this present investigation. Ritzman concluded that the growth of the lamb was not dependent upon the percentage of fat, but he was of the opinion that it depended mainly on the quantity of milk. No actual milk yields were obtained by him, but an estimation of the yields made by observation was tabulated as "high-", "good-", "fair-", and "poor-milking" ewes.

From a review of the literature it is evident that an accurate estimation of the quantity and composition of ewes' milk is necessary in order

[1] FILHOL, and JOLY. ANALYSES DU LAIT DE BREBIS APPARTENANT À DIFFÉRENTES RACES. *In* Compt. Rend. Acad. Sci. [Paris] t. 47, no. 25, p. 1013-1014. 1858.

[2] HUCHO, Hermann. UNTERSUCHUNGEN ÜBER SCHAFMILCH MIT BBESONDERER ERÜCKSICHTIGUNG DER OSTFRIESISCHEN MILCHSCHAFE. *In* Landw. Jahrb., Bd. 26, Heft 2/3, p. 496-547. 1897.

[3] FULLER, J. G., and KLEINHEINZ, Frank. ON THE DAILY YIELD AND COMPOSITION OF MILK FROM EWES OF VARIOUS BREEDS. *In* Wis. Agr. Exp. Sta. 21st Ann. Rpt. 1903/04, p. 48-50. 1904.

[4] RITZMAN, E. G. EWES' MILK: ITS FAT CONTENT AND RELATION TO THE GROWTH OF LAMBS. *In* Jour. Agr. Research, v. 8, no. 2, p. 29-36, 1 fig. 1917. Literature cited, p. 35-36.

to ascertain the factors which influence growth, since analyses of the milk of individual ewes differ widely. In the first year's work, which was preliminary in nature, five breeds of ewes were studied, an estimation of the quantity of milk given by each ewe was made every seven days, on two ewes of each breed. Chemical analyses of the samples of milk taken in 10-day periods after lambing were made for a period of 70 days. The gain of the lamb was recorded every seven days. The chief objections showing up in the preliminary work were as follows: It became evident that samples of milk for analysis and total quantities of milk ought to be taken at the same period, or as near thereto as possible. The experiment included only two ewes in each breed, and in some cases one might give an abnormally high or low milk yield, which would show unfair averages in the breed. Still another factor entered into the work. A period of 70 days proved too long, for lambs need access to grain early in their life, and as grain was fed to them this made any correlation of composition of milk and growth futile. All the above difficulties were eliminated by the following procedure adopted in this work:

PLAN OF INVESTIGATION

It was realized that any work on the study of the milk of ewes must include a number of ewes before a fair average of the milk constituents could be obtained. However, in this work the difficulty becomes very evident, for with a great number of ewes the work becomes so laborious that the use of a great number in the experiment is prohibitive. The aim was to choose three ewes which showed characteristics of the average ewe of the particular breed. This was done by starting with four ewes of each breed and continuing with the three that showed the nearest to the normal milk yield for the breed. Six breeds of ewes and three ewes from each breed were used in this experiment. The period of investigation continued for 50 days. Every 10 days after lambing the total quantity of milk was recorded, and samples of milk were taken. The weight of the lamb was taken at birth and every 10 days thereafter, from which the gain was calculated.

METHODS USED IN OBTAINING MILK SAMPLES

In determining the total milk yield of each ewe the lamb was separated from the mother ewe at 6 o'clock in the morning. At 7 it was allowed to suckle the ewe. This was done in order to start all ewes on a uniform basis. At frequent intervals during the 24-hour period, which began after the lamb suckled the ewe at 7 o'clock, the lamb was weighed, allowed to suckle, and reweighed, on a balance weighing accurately to 1 gm. The sum of the differences in the lamb's weight before and after suckling the ewe during the 24-hour period gave the total yield of milk. In this manner all the milk was obtained without causing any nervousness on the part of the ewe, and the results gave a good

representative total yield of milk. The milk samples for the analysis were obtained as follows: After the 24-hour period was concluded for the total yield of milk the lamb was kept away from the ewe until a sufficient quantity of milk was in the udder; then the lamb was allowed to suckle one side, while the other was milked dry. In this manner a uniform sample was obtained without causing undue nervousness on the part of the ewe.

CONSTITUENTS DETERMINED IN THE MILK

The samples of milk were analyzed for the following constituents: Total nitrogen, casein, albumin, fat, lactose, specific gravity, and ash. The ash was then analyzed for the calcium and phosphorus content.

METHODS USED

TOTAL NITROGEN.—A quantity of milk (approximately 5 gm.) was weighed accurately and the nitrogen determined by the Kjeldahl method.

CASEIN.—Casein was precipitated by acetic acid on a weighed quantity of milk according to the official method. The nitrogen determined by the Kjeldahl method and the results multiplied by the factor 6.38.

ALBUMIN.—After neutralizing the filtrate obtained after removing the casein, with sodium hydroxid, and adding acetic acid of the proper strength and quantity, according to the official methods,[1] the nitrogen was determined by the Kjeldahl method and the result multiplied by 6.38.

NONPROTEIN NITROGEN.—The sum of the nitrogen of the casein and albumin was subtracted from the total nitrogen. The result gave the nonprotein nitrogen.

FAT.—The fat was determined by the Babcock method.

LACTOSE.—A portion of milk (approximately 10 gm.) was weighed accurately in a flask and 25 cc. of distilled water were added. The proteins were precipitated with a sufficient quantity of colloidal ferric hydroxid as described by Hill.[2] They were then filtered off and the clear filtrate collected in a volumetric flask. The proteins were washed with distilled water until free from lactose. The combined filtrate and washings were made up to a definite volume and the lactose determined by the volumetric method of Benedict.[3] The colloidal ferric hydroxid proved to be a very efficacious clarifier, as it is very simple to use and insures thorough clarification and a clear solution.

SPECIFIC GRAVITY.—Specific gravity was determined by the Westphal balance.

ASH.—The ash was made upon composite samples of the four samples of milk by the official methods.

CALCIUM AND PHOSPHORUS.—Calcium and phosphorus were determined from the ash residues by the methods described by Richmond.[4]

DISCUSSION OF RESULTS

In Table I is found the percentage composition and total yield of milk of each ewe for the entire series taken every 10 days during a period of 24 hours. In all cases the first results upon the total weight

[1] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. REPORT OF COMMITTEE ON EDITING METHODS OF ANALYSIS. p. 287–289. Baltimore, Md., 1916. (Jour. Assoc. Off. Agr. Chem., v. 2, no. 3, pt. 2.)

[2] HILL, REUBEN L. NOTE ON THE USE OF COLLOIDAL IRON IN THE DETERMINATION OF LACTOSE IN MILK. *In* Jour. Biol. Chem., v. 20, no. 3, p. 175–176. 1915.

[3] BENEDICT, STANLEY R. THE DETECTION AND ESTIMATION OF GLUCOSE IN URINE. *In* Jour. Amer. Med. Assoc., v. 57, no. 15, p. 1193–1194. 1911.

[4] RICHMOND, HENRY DROOP. DAIRY CHEMISTRY. p. 81–82. London. 1899.

of milk were secured 10 days after the birth of the lamb. This duration of time was allowed to elapse in order to allow the milk of the ewe to become normal. Analyses of ewes' milk, made by Weiske and Kennepohl[1] at different periods, varying from 1½ hours to several days after the birth of the lamb, show that 10 days is ample time for the milk flow to assume its normal composition.

The results of the table indicate, as would naturally be assumed, that there is a decrease in the milk flow of the ewes in the 50-day period. In only one instance was this not true; that was in the case of Cotswold ewe, No. 753, which maintained not only a constant milk flow throughout the experiment, but actually showed a slight increase at the end of the 50-day period.

TABLE I.—*Quantity and composition of ewes' milk*

Breed and No. of ewes.	Sample No.	Date of sampling.	Weight of ewe.	Total quantity of milk for 24-hour period.	Specific gravity.	Casein.	Albumin.	Nonprotein nitrogen.	Fat.	Lactose.	Ash.	Ash percentage of calcium and phosphorus in ash.	
												Calcium.	Phosphorus pentoxid.
COTSWOLD			*Lbs.*	*Gm.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
2518	1	Feb. 18	190	1,931	1.029	2.44	0.91	0.076	7.2	4.79	0.87	15.15	19.92
	2	Feb. 28	184	1,980	1.033	2.47	.83	.075	8.1	4.83	.....	.....	.....
	3	Mar. 10	189	1,805	1.031	3.60	.64	.087	8.6	4.00	.....	.....	.....
	4	Mar. 20	193	1,122	1.035	3.59	.78	.062	7.4	4.93	.....	.....	.....
	5	Mar. 30	188	1,176	1.033	3.52	.83	.067	5.2	5.12	.....	.....	.....
Average			188	1,585	1.034	3.32	.81	.065	7.3	4.73	.87	15.15	19.92
753	1	Feb. 21	164	1,956	1.033	2.86	.81	.070	10.4	4.81	.77	13.24	28.24
	2	Mar. 3	159	2,141	1.032	3.01	.78	.040	8.0	5.07	.....	.....	.....
	3	Mar. 13	161	2,105	1.033	3.07	.45	.081	7.8	5.16	.....	.....	.....
	4	Mar. 23	157	1,637	1.033	3.10	.83	.051	6.4	4.79	.....	.....	.....
	5	Apr. 2	159	1,986	1.032	3.19	.54	.051	6.0	5.20	.....	.....	.....
Average			159	1,965	1.033	3.04	.68	.058	7.7	5.00	.77	15.24	28.24
8097	1	Feb. 28	159	1,555	1.035	2.82	.72	.088	7.8	4.76	.34	11.33	18.77
	2	Mar. 10	150	1,302	1.028	3.04	.52	.087	9.2	4.93	.....	.....	.....
	3	Mar. 20	147	1,113	1.033	2.94	.64	.062	7.6	4.63	.....	.....	.....
	4	Mar. 30	145	838	1.035	3.04	.85	.067	7.8	4.60	.....	.....	.....
	5	Apr. 9	149	816	1.034	2.98	.88	.059	8.2	4.22	.....	.....	.....
Average			149	1,124.8	1.033	2.96	.72	.072	8.1	4.62	.84	11.33	18.77
HAMPSHIRE													
30	1	Feb. 17	189	2,477	1.029	2.84	.72	.081	10.3	4.58	.76	12.32	22.12
	2	Feb. 27	177	2,487	1.034	2.73	.81	.072	6.2	4.80	.....	.....	.....
	3	Mar. 9	176	2,328	1.032	2.89	.48	.081	6.0	4.50	.....	.....	.....
	4	Mar. 19	165	1,845	1.035	3.62	.48	.062	7.6	4.88	.....	.....	.....
	5	Mar. 29	164	1,328	1.033	3.41	.48	.051	8.2	4.95	.....	.....	.....
Average			174	2,093	1.032	3.09	.59	.069	7.6	4.74	.76	12.32	22.12
50	1	Feb. 20	172	3,439	1.030	2.62	.91	.078	8.25	4.61	.82	14.22	24.65
	2	Mar. 2	152	2,278	1.031	3.77	.43	.059	6.85	4.53	.....	.....	.....
	3	Mar. 12	151	2,534	1.030	2.84	.36	.054	6.2	4.86	.....	.....	.....
	4	Mar. 22	136	2,300	1.031	3.36	1.36	.056	5.0	4.97	.....	.....	.....
	5	Apr. 1	139	1,848	1.035	3.07	.52	.059	3.9	4.73	.....	.....	.....
Average			150	2,479.8	1.031	3.13	.71	.061	6.0	4.74	.82	14.22	24.65

[1] WEISKE, H., AND KENNEPOHL, G. UNTERSUCHUNGEN ÜBER SCHAFMILCH UNTER VERSCHIEDENEN VERHÄLTNISSEN. *In* Jour. Landw., Jahrg. 29, p. 451–472. 1881.

TABLE I.—*Quantity and composition of ewes' milk*—Continued

Breed and No. of ewes.	Sample No.	Date of sampling.	Weight of ewe.	Total quantity of milk for 24-hour period.	Specific gravity.	Casein.	Albumin.	Nonprotein nitrogen.	Fat.	Lactose.	Ash.	Ash per centage of calcium and phosphorus in ash.	
												Calcium.	Phosphorus pentoxid.
HAMPSHIRE—con.			*Lbs.*	*Gm.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
33	1	Feb. 23	189	3,103	1.030	2.83	1.27	0.11	11.2	4.59	0.78	15.19	27.68
	2	Mar. 5	169	2,159	1.033	3.04	1.11	.09	6.0	4.83	.....	.....	.....
	3	Mar. 15	176	1,352	1.030	3.33	.59	.06	6.8	4.93	.....	.....	.....
	4	Mar. 25	168	1,505	1.032	2.88	.81	.09	6.8	4.86	.....	.....	.....
	5	Apr. 4	169	1,573	1.033	2.81	.82	.08	7.4	4.42	.....	.....	.....
Average			176	1,938.4	1.032	2.97	.92	.08	7.6	4.72	.78	15.19	27.68
SOUTHDOWN													
89	1	Feb. 23	129	1,747	1.031	3.11	1.14	.094	11.35	4.94	.76	15.39	23.78
	2	Mar. 8	127	1,383	1.027	3.24	1.04	.070	8.0	4.98	.....	.....	.....
	3	Mar. 15	128	988	1.034	4.03	.62	.067	5.6	4.97	.....	.....	.....
	4	Mar. 25	124	863	1.030	3.18	.86	.090	8.8	4.98	.....	.....	.....
	5	Apr. 4	126	753	1.034	3.60	.66	.030	7.4	4.73	.....	.....	.....
Average			127	1,146.8	1.031	3.43	.86	.070	8.2	4.92	.76	15.39	23.78
128	1	Feb. 26	110	1,754	1.040	2.51	.80	.064	5.2	5.02	.78	14.43	28.68
	2	Mar. 5	111	1,521	1.030	3.52	.88	.044	5.3	4.86	.....	.....	.....
	3	Mar. 18	111	1,368	1.033	3.73	.48	.064	8.0	4.97	.....	.....	.....
	4	Mar. 28	108	1,393	1.035	2.54	.67	.056	5.6	4.96	.....	.....	.....
	5	Apr. 7	104	1,317	1.034	4.01	.64	.078	7.0	4.59	.....	.....	.....
Average			109	1,470.6	1.034	3.26	.69	.060	6.2	4.88	.78	14.43	28.68
207	1	Mar. 10	136	1,551	1.035	3.25	.83	.120	7.6	4.01	1.19	12.22	20.96
	2	Mar. 20	133	1,262	1.035	3.72	.84	.070	8.0	4.72	.....	.....	.....
	3	Mar. 30	132	1,051	1.035	3.84	.77	.070	9.0	4.82	.....	.....	.....
	4	Apr. 9	134	895	1.037	3.76	.74	.030	7.9	3.94	.....	.....	.....
	5	Apr. 19	130	743	1.035	3.74	.91	.050	7.9	4.07	.....	.....	.....
Average			133	1,100.4	1.035	3.66	.82	.068	8.0	4.31	1.19	12.22	20.96
SHROPSHIRE													
752	1	Mar. 12	153	1,417	1.035	3.97	.65	.07	8.0	4.73	.95	17.03	27.31
	2	Mar. 22	156	1,596	1.035	2.83	.48	.08	8.4	4.83	.....	.....	.....
	3	Apr. 1	147	1,468	1.035	3.64	.65	.07	7.6	4.97	.....	.....	.....
	4	Apr. 11	142	1,037	1.035	3.66	.47	.06	7.8	4.07	.....	.....	.....
	5	Apr. 21	146	1,018	1.035	3.77	.56	.02	8.8	3.84	.....	.....	.....
Average			149	1,307.2	1.035	3.57	.56	.06	8.1	4.48	.95	17.03	27.31
366346	1	Mar. 15	167	2,602	1.034	3.98	.67	.09	6.4	4.88	.84	21.78	34.73
	2	Mar. 25	156	2,144	1.033	3.97	.72	.07	8.0	4.89	.....	.....	.....
	3	Apr. 4	156	2,148	1.034	3.95	.78	.09	6.4	4.73	.....	.....	.....
	4	Apr. 14	151	1,836	1.033	3.36	.88	.09	7.2	4.12	.....	.....	.....
	5	Apr. 24	150	1,524	1.039	3.34	.89	.07	8.0	3.82	.....	.....	.....
Average			156	2,050.8	1.034	3.72	.79	.08	7.2	4.48	.84	21.78	34.73
49	1	Mar. 14	159	1,499	1.030	3.37	.67	.066	7.2	5.10	.87	19.04	30.57
	2	Mar. 24	156	1,701	1.037	3.06	.88	.042	10.4	4.90	.....	.....	.....
	3	Apr. 3	153	1,088	1.030	3.11	.89	.092	9.6	4.73	.....	.....	.....
	4	Apr. 13	149	996	1.032	3.08	.92	.09	8.8	4.22	.....	.....	.....
	5	Apr. 23	149	924	1.034	2.98	.96	.09	9.6	3.91	.....	.....	.....
Average			153	1,241.8	1.032	3.12	.86	.076	9.1	4.57	.87	19.04	30.57
LINCOLN													
1940	1	Feb. 21	192	1,528	1.013	2.98	.81	.06	10.8	4.72	.82	16.95	28.62
	2	Mar. 3	191	1,456	1.036	2.88	.89	.043	9.0	4.71	.....	.....	.....
	3	Mar. 13	191	1,193	1.027	3.28	.56	.076	7.2	4.89	.....	.....	.....
	4	Mar. 23	186	1,191	1.025	2.98	.83	.087	8.0	4.82	.....	.....	.....
	5	Apr. 2	188	1,180	1.026	2.83	.52	.073	8.8	4.73	.....	.....	.....
Average			189	1,309.6	1.023	2.97	.72	.067	8.8	4.77	.82	16.95	28.62

TABLE I.—*Quantity and composition of ewes' milk*—Continued

Breed and No. of ewes.	Sample No.	Date of sampling.	Weight of ewe.	Total quantity of milk for 24-hour period.	Specific gravity.	Casein.	Albumin.	Nonprotein nitrogen.	Fat.	Lactose.	Ash.	Ash percentage of calcium and phosphorus in ash, Calcium.	Ash percentage of calcium and phosphorus in ash, Phosphorus pentoxid.
LINCOLN—contd.			*Lbs.*	*Gm.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
	1	Feb. 27	187	1,955	1.033	2.92	1.12	.101	7.6	4.74	0.67	15.20	27.56
	2	Mar. 9	179	1,569	1.033	3.22	.84	.062	6.0	4.72	.....	.....	.....
1913.....	3	Mar. 19	176	1,441	1.033	2.98	.58	.059	6.9	4.87	.....	.....	.....
	4	Mar. 29	176	1,274	1.035	3.39	.95	.077	6.4	4.60	.....	.....	.....
	5	Apr. 8	176	1,482	1.035	3.38	.91	.078	7.4	4.70	.....	.....	.....
Average...		.....	179	1,544.2	1.033	3.17	.88	.075	6.8	4.72	.67	15.20	27.56
	1	Feb. 24	193	1,574	1.032	2.60	1.19	.100	9.6	4.75	.80	14.73	29.13
	2	Mar. 6	180	1,313	1.032	2.67	1.26	.084	7.2	4.85	.....	.....	.....
1996.....	3	Mar. 16	182	735	1.029	3.66	.45	.037	11.4	4.93	.....	.....	.....
	4	Mar. 26	185	481	1.030	3.25	.40	.011	8.2	5.12	.....	.....	.....
	5	Apr. 5	175	506	1.032	3.35	.39	.032	8.2	4.30	.....	.....	.....
Average...		.....	183	921.8	1.031	3.10	.73	.053	8.9	4.79	.80	14.73	29.13
RAMBOUILLET													
	1	Feb. 12	136	1,918	1.033	3.63	.86	.062	10.05	4.82	.91	18.49	29.38
	2	Feb. 22	131	1,391	1.037	3.62	.75	.029	11.9	4.78	.....	.....	.....
36.....	3	Mar. 4	130	1,347	1.032	4.75	.52	.048	9.6	5.00	.....	.....	.....
	4	Mar. 14	130	1,131	1.034	4.22	1.23	.037	9.6	4.60	.....	.....	.....
	5	Mar. 24	131	1,112	1.033	4.58	.84	.070	8.2	4.68	.....	.....	.....
Average...		.....	132	1,379.8	1.034	4.16	.84	.059	9.8	4.77	.91	18.49	29.38
	1	Feb. 26	156	2,582	1.039	3.42	.98	.110	7.4	5.00	.80	16.64	31.08
	2	Mar. 8	151	2,113	1.032	3.05	.95	.019	6.8	4.97	.....	.....	.....
59.....	3	Mar. 18	149	1,766	1.040	3.40	.48	.064	6.4	5.08	.....	.....	.....
	4	Mar. 28	147	1,706	1.034	3.17	.74	.062	5.9	5.07	.....	.....	.....
	5	Apr. 7	144	1,758	1.033	3.59	.51	.029	7.0	4.56	.....	.....	.....
Average...		.....	149	1,985	1.035	3.32	.73	.056	6.7	4.93	.80	16.64	31.08
	1	Feb. 28	169	1,525	1.042	3.64	.94	.067	3.4	4.71	.86	19.64	32.47
	2	Mar. 10	167	1,262	1.030	3.45	1.27	.054	9.6	4.76	.....	.....	.....
74.....	3	Mar. 20	160	1,140	1.034	3.21	.72	.091	6.4	4.79	.....	.....	.....
	4	Mar. 30	164	880	1.037	2.96	.58	.057	8.0	4.75	.....	.....	.....
	5	Apr. 9	165	802	1.034	3.45	.86	.062	8.2	4.00	.....	.....	.....
Average...		.....	165	1,121.8	1.035	3.34	.87	.066	7.1	4.60	.86	19.64	32.47

A study of Table I brings out the fact that there is a great variability in the percentages of the constituents of ewes' milk. Not only is this true among the different breeds, but also during the lactation period of the individual. The most constant constituent in the milk of all breeds examined appears to be lactose, while fat seems to be the most variable. The difference in the percentages of fat is very marked, not only between the breeds, but during the lactation period of the individual. These observations are in harmony with the results secured by Ritzman,[1] who also found that the fat varied at different lactation periods of individual ewes and who concluded that—

> No great reliance can be placed on single tests of an individual, and that a test must either cover a larger number of periods during one lactation of an individual or that

[1] RITZMAN, E. G., 1917. OP. CIT., p. 31.

it must cover an average of a large number of individuals at one period, in order to be representative.

When the average percentages of fat for the five lactation periods of each ewe are determined and compared, the variation of fat content is not so marked, which indicates clearly the value of a number of tests rather than one single test on an individual.

TABLE II.—*Average quantity and composition of milk for each ewe and for each breed*

Breed and No. of ewe.	Average total quantity of milk for 24-hour periods.	Specific gravity.	Composition of milk.					
			Casein.	Albumin.	Non-protein.	Fat.	Lactose.	Ash.
COTSWOLD	*Gm.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
2518	1,585	1.034	3.32	0.81	0.065	7.3	4.73	0.87
753	1,965	1.032	3.04	.68	.058	7.7	5.00	.77
2097	1,124.8	1.033	2.96	.72	.072	8.1	4.62	.84
Average	1,558	1.033	3.10	.74	.065	7.7	4.78	.82
HAMPSHIRE								
50	2,479	1.031	3.13	.71	.061	6.0	4.74	.81
33	1,938.4	1.032	2.97	.92	.080	7.6	4.72	.78
30	2,093	1.032	3.09	.59	.069	7.6	4.74	.76
Average	2,170	1.032	3.06	.74	.070	7.1	4.73	.78
LINCOLN								
1940	1,309.6	1.023	2.97	.72	.067	8.8	4.77	.82
1996	921.8	1.033	3.17	.88	.077	6.8	4.72	.67
1913	1,544.2	1.025	3.10	.73	.053	8.9	4.79	.80
Average	1,258	1.027	3.08	.77	.065	8.1	4.76	.76
RAMBOUILLET								
36	1,379.8	1.039	4.16	.84	.059	9.8	4.77	.91
59	1,985	1.035	3.32	.73	.056	6.7	4.93	.80
74	1,121.8	1.035	3.34	.87	.066	7.1	4.60	.86
Average	1,495	1.036	3.60	.81	.078	7.8	4.77	.85
SOUTHDOWN								
89	1,146.8	1.031	3.43	.86	.070	8.2	4.92	.76
128	1,470.6	1.034	3.26	.69	.060	6.2	4.88	.78
807	1,100.4	1.035	3.66	.82	.068	8.0	4.31	1.19
Average	1,238	1.033	3.45	.79	.066	7.5	4.70	.91
SHROPSHIRE								
752	1,307.2	1.035	3.57	.56	.06	8.1	4.48	.95
366346	2,050.8	1.034	3.72	.79	.08	7.2	4.48	.84
49	1,241.8	1.032	3.12	.86	.07	9.1	4.57	.87
Average	1,532	1.033	3.47	.77	.07	8.1	4.50	.88

Tables II and III are given for convenience of comparison of the average yield and the average analysis of the milk for the 50-day period. Table II gives the averages for the three individual ewes of each breed, and the average of these averages is represented in Table III as the average for the breed.

TABLE III.—*Average quantity and composition of milk for each breed*

Name of breed.	Average total quantity of milk for 24-hour periods.	Specific gravity.	Composition of milk.					
			Casein.	Albumin.	Non-protein.	Fat.	Lactose.	Ash.
	Gm.		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Hampshire	2,170	1.032	3.06	0.74	0.070	7.1	4.72	0.78
Cotswold	1,558	1.033	3.10	.74	.065	7.7	4.45	.82
Shropshire	1,532	1.033	3.47	.77	.007	8.1	4.50	.88
Rambouillet	1,495	1.036	3.60	.81	.078	7.8	4.77	.85
Lincoln	1,258	1.027	3.08	.77	.065	8.1	4.76	.76
Southdown	1,238	1.033	3.45	.79	.066	7.5	4.70	.91

Table III brings out clearly the differences in milk yields for the different breeds. The Hampshire ewes in this experiment easily ranked first in quantity of milk produced, while the differences in the other five breeds were not so great.

TABLE IV.—*Initial weight of lambs and their gain during each 10-day period*

Breed and No. of ewe.	Number of lambs.	Initial weight of lambs.		Amount of weight gained by lambs each 10-day period.		
		First.	Second.	First.	Second.	Total.
SHROPSHIRE		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
752	1	4,294	.....	2,392	.....	2,392
				2,468	.....	2,468
				2,902	.....	2,902
				2,032	.....	2,032
				1,957	.....	1,957
Total gain 50 days	.....	.....	.....	11,751	.....	11,751
366346	2	3,955	4,407	2,535	2,518	5,053
				2,110	2,304	4,414
				1,372	1,452	2,824
				1,644	1,637	3,281
				1,522	1,374	2,896
Total gain 50 days	.....	.....	.....	.....	.....	18,468
49	2	3,503	3,277	1,682	1,888	3,570
				1,139	1,085	2,224
				1,395	1,023	2,418
				1,360	977	2,337
				751	761	1,511
Total gain 50 days	.....	.....	.....	.....	.....	12,060
LINCOLN						
1940	1	5,311	.....	2,507	.....	2,507
				2,269	.....	2,269
				2,296	.....	2,296
				2,089	.....	2,089
				2,111	.....	2,111
Total gain 50 days	.....	.....	.....	11,272	.....	11,272

TABLE IV.—*Initial weight of lambs and their gain during each 10-day period*—Contd.

Breed and No. of ewe.	Number of lambs.	Initial weight of lambs.		Amount of weight gained by lambs each 10-day period.		
		First.	Second.	First.	Second.	Total.
LINCOLN—continued		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1913	1	5,650	.....	3,003	.....	3,003
				2,754	.....	2,754
				2,256	.....	2,256
				2,286	.....	2,286
				1,782	.....	1,782
Total gain 50 days	.....	.....	.....	12,081	.....	12,081
1996	1	5,424	.....	2,463	.....	2,463
				2,078	.....	2,078
				[a] 844	.....	[a] 844
				696	.....	696
				702	.....	702
Total gain 50 days	.....	.....	.....	6,783	.....	6,783
RAMBOUILLET						
36	1	5,085	.....	2,189	.....	2,189
				3,114	.....	3,114
				2,117	.....	2,117
				2,395	.....	2,395
				1,681	.....	1,681
Total gain 50 days	.....	.....	.....	11,496	.....	11,496
59	2	4,181	4,181	2,526	2,230	4,756
				249	1,969	2,218
				1,140	1,722	2,862
				770	1,681	2,451
				1,612	1,423	3,035
Total gain 50 days	.....	.....	.....	.....	.....	16,092
74	2	3,616	3,390	1,427	937	2,364
				542	598	1,140
				992	780	1,772
				755	795	1,550
				1,012	1,227	2,239
Total gain 50 days	.....	.....	.....	.....	.....	9,065
COTSWOLD						
2518	2	4,633	4,520	2,431	2,318	4,749
				1,527	1,587	3,114
				1,305	1,566	2,871
				1,260	1,363	2,623
				1,222	1,152	2,374
Total gain 50 days	.....	.....	.....	.....	.....	15,731
753	1	4,068	.....	3,696	.....	3,696
				3,549	.....	3,549
				2,976	.....	2,976
				2,995	.....	2,995
				2,191	.....	2,191
Total gain 50 days	.....	.....	.....	15,407	.....	15,407

[a] Lamb sick, did not thrive.

TABLE IV.—*Initial weight of lambs and their gain during each 10-day period*—Contd.

Breed and No. of ewe.	Number of lambs.	Initial weight of lambs.		Amount of weight gained by lambs each 10-day period.		
		First.	Second.	First.	Second.	Total.
COTSWOLD—continued		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
2097	1	5,650	.....	2,945	.....	2,945
				1,861	.....	1,861
				1,561	.....	1,561
				1,646	.....	1,646
				1,796	.....	1,796
Total gain 50 days				9,809	.....	9,809
HAMPSHIRE						
30	2	5,424	3,503	1,932	1,679	3,611
				2,919	2,479	5,398
				1,937	1,149	3,086
				2,308	1,527	3,835
				1,934	822	2,756
Total gain 50 days						18,686
50	2	4,407	4,859	3,735	3,338	7,073
				2,213	2,202	4,415
				1,598	2,073	3,681
				2,061	2,275	4,336
				1,416	2,315	3,731
Total gain 50 days						23,236
33	1	4,294	.....	6,349	.....	6,349
				3,814	.....	3,814
				2,769	.....	2,769
				2,835	.....	2,835
				2,317	.....	2,317
Total gain 50 days				18,084	.....	18,084
SOUTHDOWN						
89	1	4,294	.....	2,764	.....	2,764
				2,752	.....	2,752
				2,239	.....	2,239
				1,864	.....	1,864
				982	.....	982
Total gain 50 days				10,601	.....	10,601
128	1	4,068	.....	2,995	.....	2,995
				2,443	.....	2,443
				2,592	.....	2,592
				1,950	.....	1,950
				1,789	.....	1,789
Total gain 50 days				11,769	.....	11,769
207	2	3,616	3,503	1,503	1,555	3,058
				983	1,280	2,263
				815	1,207	2,022
				886	1,524	2,410
				1,247	1,483	2,730
Total gain 50 days						12,483

In Table IV data are given on the initial weight of the lamb or lambs and the gain in weight every 10 days during the period of the experiment. The total gain is also included.

Table V is a combination of the results on total milk yield and the total constituents of the milk, expressed in grams, calculated from the average percentages secured on the 50-day period, and also data on the total gain in weight of the lambs.

Table VI gives the averages of the above constituents for each breed.

TABLE V.—*Relation of Milk Constituents of Individual Ewes to Growth of Lambs*

Breed and No. of ewe.	Total quantity of milk.	Total casein.	Total albumin.	Total non-proteids.	Total fat.	Total lactose	Total ash.	Weight at birth.		Number of lambs to ewe.	Total growth.
								Lamb No. 1.	Lamb No. 2.		
HAMPSHIRE	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*Gm.*
30	104,650	3,237	617	72	7,953	4,960	795	5,424	3,505	[a] 2	21,764
50	123,950	3,897	880	76	7,437	5,974	1,004	4,407	4,859	2	23,236
33	96,920	2,878	891	77	7,375	4,574	756	4,294	.....	1	18,084
Average	108,506.6	3,331	796	75	7,585	5,126	852	.....	.....	.....	21,028
COTSWOLD											
753	98,250	2,987	668	57	7,565	4,912	756	4,068	.....	1	15,407
2518	79,250	2,631	642	52	5,785	3,748	689	4,633	4,520	2	17,731
2097	56,240	1,664	405	40	4,555	2,588	472	5,650	.....	1	9,809
Average	77,913.3	2,427	572	50	5,968	3,749	639	.....	.....	.....	14,318
RAMBOUILLET											
36	68,990	2,870	579	41	6,761	3,280	627	5,085	3,842	[b] 1	13,078
59	99,250	3,295	705	56	6,650	4,913	794	4,181	4,181	2	15,322
74	56,090	1,873	487	37	3,982	2,680	482	3,616	3,390	2	9,065
Average	75,110	2,677	590	45	5,797	3,624	634	.....	.....	.....	12,488
LINCOLN											
1940	65,450	1,944	471	44	5,650	3,122	537	5,311	.....	1	11,272
1913	77,210	2,393	564	41	6,871	3,698	618	5,424	.....	1	12,081
1996	46,090	1,461	406	35	3,135	2,176	309	5,650	.....	1	6,783
Average	63,250	1,933	480	40	5,219	2,999	388	.....	.....	.....	10,045
SOUTHDOWN											
89	57,340	1,967	493	40	4,702	2,821	435	4,294	.....	1	10,601
128	73,530	2,397	507	51	5,559	3,588	573	4,068	.....	1	11,769
207	55,020	2,013	451	37	4,401	2,371	654	3,616	3,503	2	12,483
Average	61,963.3	2,136	484	43	4,554	2,926	554	.....	.....	.....	11,618
SHROPSHIRE											
753	65,360	2,333	366	39	5,294	2,928	621	4,294	.....	1	11,751
366346	102,540	3,814	810	82	7,382	4,594	861	3,955	4,407	2	18,468
49	62,090	1,937	534	43	5,650	2,837	540	3,503	3,277	2	12,060
Average	76,663.3	2,695	570	55	6,109	3,452	675	.....	.....	.....	14,093

[a] Ewe had triplets, one was taken away on ninth day.
[b] Lamb had leg broken on ninth day and was removed.

In a comparison of the total quantity of milk constituents and the total growth of the lambs there is one disturbing factor. In all breeds, with the exception of the Lincoln, twins were born to one or more ewes in each breed, and in one case triplets. The three Lincoln ewes all gave birth to single lambs. It is obvious that in comparison of quantity

of milk and growth of lambs the best experimental results in this investigation would have been obtained if all ewes were allowed to raise only one lamb. In future work it is hoped that this condition may be fulfilled. However, many factors prevented such an arrangement. At the time of this investigation, which is an outgrowth of a more extended investigation on sheep, it was desired to make the work correspond as closely as possible to the actual conditions found in sheep husbandry, and other data were collected besides those included in this paper.

TABLE VI.—*Relation of Milk Constituents of Breeds to Growth of Lambs*

Breed.	Total quantity of milk.	Total casein	Total albumin.	Total non-proteids.	Total fat.	Total lactose.	Total ash.	Number of lambs to ewes.	Total growth.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*Gm.*
Hampshire	108,506.6	3,331	796	75	7,585	5,126	852	5	21,028
Cotswold	77,913.3	2,427	572	50	5,968	3,749	639	4	14,318
Shropshire	76,636.3	2,695	570	55	6,107	3,452	674	5	14,093
Rambouillet	75,110	2,677	590	45	5,797	3,624	634	5	12,488
Lincoln	63,250	1,933	480	40	5,219	2,999	388	3	10,045
Southdown	61,963.3	2,136	484	43	4,554	2,926	554	4	11,618

It is quite evident that twin lambs, given a sufficient quantity of milk, will make a greater total gain than a single lamb, provided their initial weights correspond and they are equally strong at birth. A certain amount of milk is essential for the growth of a lamb, but on the other hand there is a limit to the amount of milk that an animal can assimilate. Therefore, two lambs, given a sufficient quantity of milk, will have an advantage in total gain over a single lamb. The single lamb, however, is generally larger than either of the twin lambs at birth, but from an economical standpoint it is obvious that twins are more desirable in the flock than singles.

A compilation of the data on the Hampshire breed shows the single lamb of ewe No. 33 gained nearly as much in the same period of time as the twin lambs of ewe No. 30. A comparison of the total yield of milk shows ewe No. 33 produced slightly less than ewe No. 30.

In the Cotswold breed we have ewe No. 753 giving more than either of the other two ewes, and the single lamb has made a gain almost equal to the gain of the twin lambs of ewe No. 2518. Ewe No. 2518 has twin lambs, and their total gain is only slightly greater than the single lamb of ewe No. 753. The third ewe, No. 2097, shows a smaller milk yield than ewe No. 2518 with the twin lambs, and the gain of her single lamb is a little more than one-half as much as the total gain of the twin lambs. In the Rambouillet and Southdown breeds we find the total gain in weight of the lambs is proportional to the amount of milk consumed. In the Lincoln breed, the only breed where there are three single lambs, their gain in weight is also proportional to the quantity of milk consumed. However, the lamb belonging to the Lincoln ewe, No. 1996, became sick at the end of 20 days and did not thrive thereafter.

It appears from this experiment that the greatest factor in growth is quantity of milk; hence, a high-milk-producing ewe is more valuable than a low one. The inherited capacity for growth, however, must not be overlooked. As to the relative merits of the breeds, it is not the purpose of this investigation to enter upon a discussion. To draw conclusions upon such a small number of ewes in each breed would be unfair. It was the aim of this experiment to make the investigation as fair as possible to all breeds studied, and the authors desire to emphasize clearly the fact that results upon the different breeds are given wholly as an attempt to correlate milk yields, their composition, and their relation to growth. The results are not given with an idea of comparing the desirability or undesirability of the breeds included in this experiment, but rather for the purpose of presenting to the farmer and student information in regard to features of certain well-known breeds that have to do with utility and adaptation to certain specific purposes. For example, the man interested in the growth of lambs for early marketing would be interested in a breed that by its yield of milk, and possibly certain other factors, made the greatest average growth of lambs. Another purpose of the experiment is to stimulate the interest of investigators and students of animal breeding in the field for the improvement of certain breeds with reference to factors having to do with profit for the grower. There might even be room for a new breed that would possess all the desirable and highly useful factors of some of the breeds included in this experiment.

SEED DISINFECTION BY FORMALDEHYDE VAPOR

[PRELIMINARY REPORT]

By CECIL C. THOMAS

Pathological Inspector, Federal Horticultural Board, United States Department of Agriculture

INTRODUCTION

The continual introduction of plants by the Department of Agriculture, chiefly by means of seeds, from all parts of the world, and the constant danger of allowing little-known or serious diseases to enter thereon, emphasize the necessity for a study of the methods of seed disinfection. There are few data on this subject except in the case of cereals and for a small number of seeds used in physiological experiments.

The pathological inspectors of the Federal Horticultural Board have encountered many difficulties in treating hundreds of lots of seeds of widely varying types and quantities with the various liquid treatments in common use. Most of the treatments recommended and used at the present time require dipping or soaking in a water solution of some fungicide or germicide. The seeds, therefore, remain wet for a longer or shorter period, depending on the treatment given and the method of drying.

Some seeds like wheat and rye absorb water slowly and can be dried without much injury, while seeds like the various members of the mustard family absorb water very readily and with even a very brief treatment swell sufficiently to break the seed coat and allow the cotyledons to fall apart, thus destroying the seed.

Light seeds such as are found in many of the grasses present another problem for the wet treatment. It is very difficult to give them anything like a uniform treatment because of the difficulty of wetting them and keeping them under the liquid. Seeds such as flax, which have a mucilaginous covering, present still another difficulty for wet treatments.

The large number of shipments and the great variety of seeds passing through the quarantine inspection house of the Federal Horticultural Board, United States Department of Agriculture, that need to be treated render desirable the adoption of a method of treatment which will obviate wetting and drying. An attempt, therefore, is being made to develop a treatment of this type with formaldehyde vapor. While it is far from being perfected, it seems desirable to make a preliminary report on some of the results obtained.

Journal of Agricultural Research, Washington, D. C.
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Vol. XVII, No. 1
Apr. 15, 1919
Key No. O—3

The pathological inspectors of the Federal Horticultural Board have treated several hundred lots of seeds each year in the liquid treatments commonly recommended and have found the formaldehyde solutions the best for the greatest number of cases. Formaldehyde is also known to be a very efficient germicide when used in the form of a vapor as a disinfectant for contagious human diseases.

For the above reasons formaldehyde vapor has been selected for this work.

APPARATUS

A galvanized iron can (fig. 1, A) having a capacity of approximately 130,000 cc. was used. After introducing the seeds and organisms,

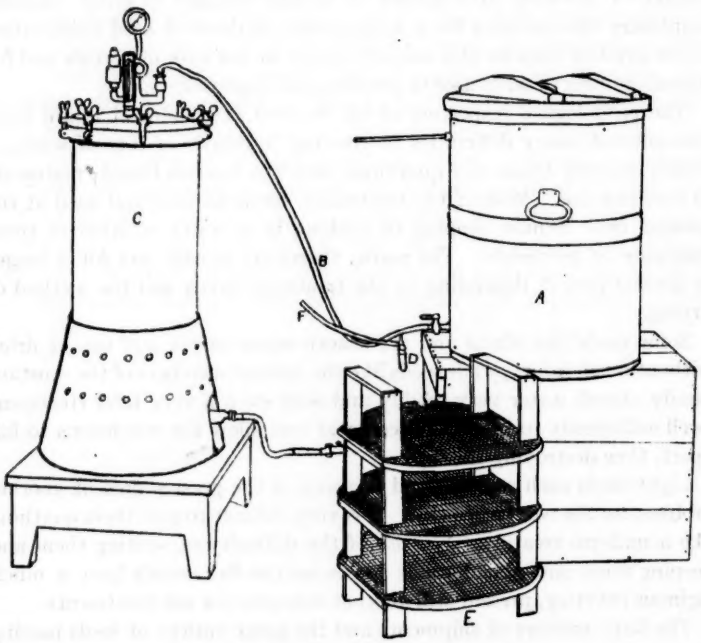

Fig. 1—Formaldehyde-vapor disinfecting apparatus

steam was added through a rubber tube (B) from an autoclave (C). The formaldehyde solution (Shoemaker and Busch, U. S. P. VIII, 40 per cent by volume of formic aldehyde) was diluted one to one with water to give a greater volume of liquid. This formaldehyde solution was introduced through an atomizer (D) with the aid of compressed air (F), the nozzle of the atomizer being inserted in the rubber tube (B), through which the steam passes as it enters the can. This insertion was made as close to the can as possible. Steam was first introduced and then the compressed-air tube was attached to the atomizer and the formaldehyde solution was forced in while the steam was still entering.

The finely atomized formaldehyde solution thus enters the can and is carried to all parts of it with the steam. Condensation takes place on the surface of the seeds, forming a thin film of moisture about each seed in which the formaldehyde may act, and as this film evaporates the gas is freed.

A frame (E) containing three wire shelves was used inside the can, and the seeds were placed in porcelain dishes on these shelves.

EXPERIMENTS

There are two distinct phases of this problem: (1) The effect on the seeds and (2), the effect on the fungi and bacteria.

Table I gives the result of a series of treatments of a number of different seeds. The formaldehyde solution, before dilution, was used at the rate of 10 ounces per 1,000 cubic feet to procure the results given in the second, third, and fourth columns and for the time indicated. The results given in the sixth column are for formaldehyde used at the rate of 30 ounces per 1,000 cubic feet for 2 hours.

The germination percentages given in this table are an average of the results obtained by the Seed Laboratories, Bureau of Plant Industry, United States Department of Agriculture, from germinating two samples of 100 seeds in each case.

TABLE I.—*Effect of formaldehyde vapor of different strengths for varying lengths of time on the germination of seed*

Seed.	Checks.	Strength formalin.				
		10 ounces per 1,000 cubic feet.			Checks.	30 ounces per 1,000 cubic feet (2 hours).
		1 hour.	2 hours.	3 hours.		
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Alfalfa (C. I. 44)[1]	95.5	92	93.5	93.5	96	89.5
Barley (C. I. 25)[1]	92.5	94.5	93.5	94	90.5	92.5
Beet	71.5	58.5	64	66	66	68.5
Carrot, Oxheart	69.5	72.5	66.5	83.5	72.5	79
Clover, Crimson	70.5	69	75.5	65	69	64
Corn, Miner's Yellow Dent	96.5	95.5	96.5	97	97	98
Field Pea	93.5	94.5	89.5	93	93.5	89
Flax	94.5	92	93.5	93	86	85
Lettuce	97.5	97	98	99	99	99
Millet	94.5	92.5	93	92.5	81.5	88.5
Muskmelon, Rocky Ford	87	82.5	88	95.5	95	93.5
Natal Grass	1.5	3.5	3	4.5	3	0
Oats (C. I. 541-4)[1]	98	98	99	97	98	98
Orchard Grass	64.5	63.5	72.5	73.5	76.5	61.5
Radish, Icicle	97	98.5	97	98	96	96.5
Rice (C. I. 1561)[1]	93.5	94	96	92	93.5	95
Rye (C. I. 138)[1]	83.5	84.5	89.5	87	83	86
Soy Bean	95.5	96	96.5	98	99	97.5
Sudan Grass	84	90.5	87.5	84	80	84.5
Wheat, Blue Stem (C. I. 1912-11)[1]	57.5	61.5	59.5	55.5	62	61.5

[1] These are accession numbers of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, from which some of the seeds were obtained.

Apparently there was little or no injury in any case. As shown by the percentage of germination in the checks the killing of all seeds in the case of Natal grass where 30 ounces per thousand cubic feet for 2 hours was used probably was due to the low vitality of the seeds.

A number of experiments have been conducted with fungi and bacteria in which they were treated with various amounts of the formaldehyde vapor and for different lengths of time. Five different organisms, *Monilia fructigena*, *Colletotrichum gloeosporioides*, *Fusarium vasinfectum*, *Ascochyta* sp., and *Bacillus caratovorus* were used in the following experiments.

The spores were exposed to the treatment in four different ways: (1) Three drops from a cloudy water suspension were placed on the bottom of a sterile petri dish with a sterile platinum loop and dried before treating. (2) Three drops of the suspension were placed in a dish as above and the dish was placed in the treating chamber before the drops had dried. (3) The drops from the suspension were placed on sterile cover glasses and these were then placed in sterile petri dishes and treated. After treatment the cover glasses were removed to another petri dish in order to avoid a chance of getting any great amount of formaldehyde into the culture medium when the plates were poured. (4) Masses of dry spores were used. The masses of spores were placed on cover glasses by smearing with a platinum loop containing an abundance of spores taken from the surface of a pure culture. These cover glasses were then handled as described under the third method.

The checks were made in the same way as the plates used in the first method except that they were not treated in any way. After the treatments all plates were poured, using potato agar. They were kept under observation for from a week to 10 days.

The use of different amounts of formaldehyde solution and changes in duration of the treatment show that under the conditions described 10 ounces of standard formaldehyde solution per 1,000 cubic feet for 1 hour will kill the organisms used when they are exposed in a thin film. When a mass of spores is used, more time is necessary to kill them.

The masses of organisms or spores in the case of *Bacillus caratovorus* and Monilia were killed when formaldehyde was used at the rate of 10 ounces per 1,000 cubic feet for 2 hours; Ascochyta spores in mass were killed when formaldehyde was used at the rate of 20 ounces per 1,000 cubic feet for 1 hour; but 20 ounces per 1,000 cubic feet for 2 hours was necessary to kill the masses of spores of Colletotrichum. Fusarium proved to be the most resistant, and a test was made using the spores of four different species of Fusarium in masses. Formaldehyde was used at the rate of 30 ounces per 1,000 cubic feet for 2 hours, and in all cases growth occurred.

Following the above experiments some work was undertaken to determine the effect of formaldehyde vapor on the fungous spores and bacteria borne on the surface of seeds. Five seeds of each of the various kinds

used above were placed in a series of sterile petri dishes, two sets of each kind of seeds being used. One set of dishes was held untreated as a check and the other set was given the vapor, using formaldehyde solution at the rate of 20 ounces per 1,000 cubic feet for 2 hours. The plates were all poured in the usual way and observations were made for several days. This experiment was repeated three times, and very promising results were secured. Alfalfa, carrot, clover, field pea, flax, lettuce, millet, muskmelon, radish, and soy beans were free from fungi when treated, but fungi were present in abundance in all the checks, except flax.

In practically all cases, whether treated or untreated, a few bacteria developed on the plates, but the treated plates showed very few colonies, while the checks showed a great many.

In the case of barley, oats, corn, rye, rice, and wheat no growth appeared for two or three days in the treated plates, while the untreated plates had an abundance of growth within a day or two. This difference may be due to an inhibiting effect on the part of the vapor, but it seems more probable that it is due to the fact that the surface spores and mycelia were killed in the case of the treated seeds and that the appearance of fungi a day or two later is due to the growth of mycelium from within. The fungi appearing in such cases were species of Fusarium and Alternaria.

It seemed desirable to determine in so far as possible what fungi are present on the seeds passing through the inspection house and at the same time get some additional information as to what effect the vapor treatment would have on these organisms under actual working conditions. A chance was also afforded to study the effect of the vapor treatment, in a very limited way, on germination.

In this work five seeds of the material to be tested were removed before treatment and five after treatment. These seeds were placed in sterile petri dishes and treated in the usual way. The plants were under observation for several days. Bean seeds were used more than any other, inasmuch as a large number of shipments of beans happened to be coming in from South America.

Out of the 86 different samples of beans treated and studied 9 seemed to be retarded from one to two days in germination, while 8 were accelerated slightly, but in no case was there any apparent injury. The remainder of the samples did not seem to be affected one way or the other, so far as their germination was concerned. There was very marked reduction in the number of fungi and bacteria present in the treated samples as compared with the untreated. In taking samples of this kind, average seeds were selected, and as a result some of the beans were diseased and probably had internal mycelium, as in the case of Colletotrichum, thus making it impossible to render them absolutely free from fungi without killing them.

Sixteen different fungi were found in these samples, including Fusarium, Alternaria, and Colletotrichum, species of all three of which are known to cause serious bean diseases.

In all of the experiments set forth above, only a few seeds were included in each sample and inasmuch as formaldehyde vapor is known to be lacking in penetration it seemed desirable to try the treatment of a larger quantity of seeds.

A shipment of poppy and Cryptotaenia seed afforded an opportunity to try the treatment of a larger quantity of seeds than previously had been attempted. The samples used were of sufficient size to cover the bottom of the dish in which the seeds were treated to a depth of three-fourths of an inch. After treating the seeds a sample was taken from the surface and then the seeds were carefully removed from the surface to a depth of about one-half inch where another sample was taken. These samples were plated out and the samples taken one-half inch below the surface showed fully as many colonies of fungi and bacteria as did the untreated samples while those taken from the surface showed no fungi and a marked reduction in bacteria. This experiment shows the lack of penetration of formaldehyde vapor.

A comparison of the formaldehyde vapor and a 2 per cent formalin solution was made. Ten different kinds of seeds were used and three samples, each consisting of five seeds, of each of the different kinds of seeds were made and placed in sterile petri dishes. The first set of each was retained as a check; the second was treated with 2 per cent formalin for 10 minutes and then washed with sterilized water twice, while the third was given formaldehyde gas at the rate of 20 ounces of formalin per 1,000 cubic feet for 2 hours. After treatment all the plates, including the checks, were poured and kept under observation for several days. In all cases the 2 per cent formalin sample stood intermediate between the checks and those treated with formaldehyde vapor. The vapor-treated samples were remarkably free from fungi and bacteria. In fact only the wheat, rice, and rye samples had any fungi present, and there was much less growth in these than in the checks or in those treated with 2 per cent formalin. One of the noticeable things in this experiment was that in all the plates treated with the vapor there was a very marked reduction in the number of bacterial colonies, as compared with the checks, while the samples treated with 2 per cent formalin showed little or no reduction in the number of bacterial colonies, as compared with the checks It should be stated in connection with these experiments that different lots of seeds or a change in any one of the many factors concerned in all probability would bring about a change in the results obtained.

Several hundred lots of seeds have been treated with 20 ounces of formaldehyde per 1,000 cubic feet. Subsequent plating in agar has shown that molds and other fungi rarely appear in these plates if the

seeds are sound, whereas the untreated checks seldom fail to develop several colonies.

The work thus far necessarily has been limited to a few fungi and a few seeds, but there is an almost unlimited field here that needs investigation if efficient and satisfactory results are to be obtained in the disinfection of seeds.

CONCLUSION

1. The use of liquids for disinfection is unsatisfactory for many kinds of seeds.

2. A number of species of fungi and bacteria are killed when treated for 2 hours with 20 ounces of formaldehyde vaporized under the conditions described.

3. This same treatment is not injurious to any of the seeds tested.

4. The experiments completed indicate that the formaldehyde gas treatment described is a very efficient means of seed disinfection.

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